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COMPOSITIONS FOR USE IN IDENTIFICATION OF BACTERIA

STATEMENT OF GOVERNMENT SUPPORT

[01] This invention was made with United States Government support under CDC contract RO1 CJ000099-01. The United States Government has certain rights in the invention.

FIELD OF THE INVENTION

[02] The present invention provides compositions, kits and methods for rapid identification and quantification of bacteria by molecular mass and base composition analysis.

BACKGROUND OF THE INVENTION

- [03] A problem in determining the cause of a natural infectious outbreak or a bioterrorist attack is the sheer variety of organisms that can cause human disease. There are over 1400 organisms infectious to humans; many of these have the potential to emerge suddenly in a natural epidemic or to be used in a malicious attack by bioterrorists (Taylor et al. Philos. Trans. R. Soc. London B. Biol. Sci., 2001, 356, 983-989). This number does not include numerous strain variants, bioengineered versions, or pathogens that infect plants or animals.
- [04] Much of the new technology being developed for detection of biological weapons incorporates a polymerase chain reaction (PCR) step based upon the use of highly specific primers and probes designed to selectively detect certain pathogenic organisms. Although this approach is appropriate for the most obvious bioterrorist organisms, like smallpox and anthrax, experience has shown that it is very difficult to predict which of hundreds of possible pathogenic organisms might be employed in a terrorist attack. Likewise, naturally emerging human disease that has caused devastating consequence in public health has come from unexpected families of bacteria, viruses, fungi, or protozoa. Plants and animals also have their natural burden of infectious disease agents and there are equally important biosafety and security concerns for agriculture.
- [05] A major conundrum in public health protection, biodefense, and agricultural safety and security is that these disciplines need to be able to rapidly identify and characterize infectious agents, while there is no existing technology with the breadth of function to meet this need. Currently used methods for identification of bacteria rely upon culturing the bacterium to effect isolation from other

organisms and to obtain sufficient quantities of nucleic acid followed by sequencing of the nucleic acid, both processes which are time and labor intensive.

- [06] Mass spectrometry provides detailed information about the molecules being analyzed, including high mass accuracy. It is also a process that can be easily automated. DNA chips with specific probes can only determine the presence or absence of specifically anticipated organisms. Because there are hundreds of thousands of species of benign bacteria, some very similar in sequence to threat organisms, even arrays with 10,000 probes lack the breadth needed to identify a particular organism.
- [07] The present invention provides oligonucleotide primers and compositions and kits containing the oligonucleotide primers, which define bacterial bioagent identifying amplicons and, upon amplification, produce corresponding amplification products whose molecular masses provide the means to identify bacteria, for example, at and below the species taxonomic level.

SUMMARY OF THE INVENTION

- [08] The present invention provides compositions, kits and methods for rapid identification and quantification of bacteria by molecular mass and base composition analysis.
- [09] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 456,
- [10] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1261.
- [11] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 456 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1261.
- [12] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 288.
- [13] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1269.

[14] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 288 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1269.

- [15] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 698.
- [16] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1420.
- [17] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 698 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1420.
- [18] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 217.
- [19] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1167
- [20] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 217 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1167.
- [21] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 399.
- [22] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1041.
- [23] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 399 and an

oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1041.

- [24] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 430.
- [25] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1321.
- [26] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 430 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1321.
- [27] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 174.
- [28] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 853.
- [29] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 174 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 853.
- [30] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 172.
- [31] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1360.
- [32] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 172 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1360.

[33] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 456 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1261.

- [34] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 456 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1261 and further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 288:1269, 698:1420, 217:1167, 399:1041, 430:1321, 174:853, and 172:1360.
- [35] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 681.
- [36] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1022.
- [37] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 681 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1022
- [38] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 315.
- [39] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1379.
- [40] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 315 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1379.

[41] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 346.

- [42] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 955.
- [43] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 346 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 955
- [44] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 504.
- [45] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1409.
- [46] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 504 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1409.
- [47] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 323.
- [48] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1068.
- [49] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 323 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1068.
- [50] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 479.

[51] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 938.

- [52] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 479 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 938.
- [53] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 681 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1022.
- [54] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 681 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1022 and further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 315:1379, 346:955, 504:1409, 323:1068, 479:938.
- [55] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 583.
- [56] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 923.
- [57] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 583 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 923.
- [58] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 454.

[59] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1418.

- [60] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 454 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1418.
- [61] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 250.
- [62] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 902.
- [63] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 250 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 902.
- [64] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 384.
- [65] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 878.
- [66] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 384 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 878.
- [67] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 694.
- [68] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1215.

[69] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 694 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1215.

- [70] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 194.
- [71] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1173.
- [72] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 194 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1173.
- [73] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 375.
- [74] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 890.
- [75] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 375 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 890.
- [76] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 656.
- [77] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1224.
- [78] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID

NO: 656 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1224.

- [79] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 618.
- [80] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1157.
- [81] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 618 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1157.
- [82] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 302.
- [83] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 852.
- [84] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 302 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 852.
- [85] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 199.
- [86] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 889.
- [87] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 199 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 889.

[88] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 596.

- [89] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1169.
- [90] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 596 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1169.
- [91] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 150.
- [92] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1242.
- [93] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 150 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1242.
- [94] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 166.
- [95] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1069.
- [96] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 166 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1069.
- [97] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 166.

[98] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1168.

- [99] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 166 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1168.
- [100] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 583 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 923 and further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 454:1418, 250:902, 384:878, 694:1215, 194:1173, 375:890, 656:1224, 618:1157, 302:852, 199:889, 596:1169, 150:1242, 166:1069 and 166:1168.
- [101] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 437.
- [102] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1137.
- [103] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 437 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1137.
- [104] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 530.
- [105] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 891.
- [106] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID

NO: 530 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 891.

- [107] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 474.
- [108] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 869.
- [109] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 474 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 869.
- [110] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 268.
- [111] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1284.
- [112] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 268 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1284.
- [113] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 418.
- [114] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1301.
- [115] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 418 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1301.

[116] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 318.

- [117] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1300.
- [118] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 318 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1300.
- [119] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 440.
- [120] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1076.
- [121] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 440 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1076.
- [122] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 219.
- [123] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1013.
- [124] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 219 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1013.
- [125] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 437 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence

identity with SEQ ID NO: 1137 and further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 530:891, 474:869, 268:1284, 418:1301, 318:1300, 440:1076 and 219:1013.

- [126] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 325.
- [127] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1163.
- [128] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 325 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1163.
- [129] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 278.
- [130] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1039.
- [131] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 278 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1039.
- [132] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 465.
- [133] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1037.
- [134] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID

NO: 465 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1037.

- [135] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 148.
- [136] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1172.
- [137] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 148 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1172.
- [138] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 190.
- [139] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1254.
- [140] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 190 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1254.
- [141] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 266.
- [142] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1094.
- [143] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 266 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1094.

[144] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 508.

- [145] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1297.
- [146] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 508 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1297.
- [147] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 259.
- [148] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1060.
- [149] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 259 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1060.
- [150] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 325 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1163 and further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 278:1039: 465:1037, 148:1172, 190:1254, 266:1094, 508:1297 and 259:1060.
- [151] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 376.
- [152] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1265.

[153] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 376 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1265.

- [154] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 267.
- [155] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1341.
- [156] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 267 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1341.
- [157] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 705.
- [158] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1056.
- [159] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 705 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1056.
- [160] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 710.
- [161] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1259.
- [162] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID

NO: 710 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1259.

- [163] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 374.
- [164] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1111.
- [165] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 374 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1111.
- [166] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 545.
- [167] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 978.
- [168] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 545 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 978.
- [169] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 249.
- [170] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1095.
- [171] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 249 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1095.

[172] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 195.

- [173] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1376.
- [174] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 195 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1376.
- [175] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 311.
- [176] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1014.
- [177] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 311 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1014.
- [178] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 365.
- [179] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1052.
- [180] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 365 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1052.
- [181] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 527.

[182] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1071.

- [183] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 527 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1071.
- [184] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 490.
- [185] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEO ID NO: 1182.
- [186] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 490 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1182.
- [187] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 376 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1265 and further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 267:1341, 705:1056, 710:1259, 374:1111, 545:978, 249:1095, 195:1376, 311:1014, 365:1052, 527:1071 and 490:1182.
- [188] In some embodiments, either or both of the primers of a primer pair composition contain at least one modified nucleobase such as 5-propynyluracil or 5-propynylcytosine for example.
- [189] In some embodiments, either or both of the primers of the primer pair comprises at least one universal nucleobase such as inosine for example.
- [190] In some embodiments, either or both of the primers of the primer pair comprises at least one non-templated T residue on the 5'-end.

[191] In some embodiments, either or both of the primers of the primer pair comprises at least one non-template tag.

- [192] In some embodiments, either or both of the primers of the primer pair comprises at least one molecular mass modifying tag.
- [193] In some embodiments, the present invention provides primers and compositions comprising pairs of primers, and kits containing the same, and methods for use in identification of bacteria. The primers are designed to produce amplification products of DNA encoding genes that have conserved and variable regions across different subgroups and genotypes of bacteria.
- [194] Some embodiments are kits that contain one or more of the primer pair compositions. In some embodiments, each member of the one or more primer pairs of the kit is of a length of 14 to 35. nucleobases and has 70% to 100% sequence identity with the corresponding member from any of the primer pairs listed in Table 2.
- [195] Some embodiments of the kits contain at least one calibration polynucleotide for use in quantitiation of bacteria in a given sample, and also for use as a positive control for amplification.
- [196] Some embodiments of the kits contain at least one anion exchange functional group linked to a magnetic bead.
- [197] In some embodiments, the present invention also provides methods for identification of bacteria. Nucleic acid from the bacterium is amplificationing the primers described above to obtain an amplification product. The molecular mass of the amplification product is measured. Optionally, the base composition of the amplification product is determined from the molecular mass. The molecular mass or base composition is compared with a plurality of molecular masses or base compositions of known analogous bacterial identifying amplicons, wherein a match between the molecular mass or base composition and a member of the plurality of molecular masses or base compositions identifies the bacterium. In some embodiments, the molecular mass is measured by mass spectrometry in a modality such as electrospray ionization (ESI) time of flight (TOF) mass spectrometry or ESI Fourier transform ion cyclotron resonance (FTICR) mass spectrometry, for example. Other mass spectrometry techniques can also be used to measure the molecular mass of bacterial bioagent identifying amplicons.

[198] In some embodiments, the present invention is also directed to a method for determining the presence or absence of a bacterium in a sample. Nucleic acid from the sample is amplified using the composition described above to obtain an amplification product. The molecular mass of the amplification product is determined. Optionally, the base composition of the amplification product is determined from the molecular mass. The molecular mass or base composition of the amplification product is compared with the known molecular masses or base compositions of one or more known analogous bacterial bioagent identifying amplicons, wherein a match between the molecular mass or base composition of the amplification product and the molecular mass or base composition of one or more known bacterial bioagent identifying amplicons indicates the presence of the bacterium in the sample. In some embodiments, the molecular mass is measured by mass spectrometry.

[199] In some embodiments, the present invention also provides methods for determination of the quantity of an unknown bacterium in a sample. The sample is contacted with the composition described above and a known quantity of a calibration polynucleotide comprising a calibration sequence. Nucleic acid from the unknown bacterium in the sample is concurrently amplified with the composition described above and nucleic acid from the calibration polynucleotide in the sample is concurrently amplified with the composition described above to obtain a first amplification product comprising a bacterial bioagent identifying amplicon and a second amplification product comprising a calibration amplicon. The molecular masses and abundances for the bacterial bioagent identifying amplicon and the calibration amplicon are determined. The bacterial bioagent identifying amplicon is distinguished from the calibration amplicon based on molecular mass and comparison of bacterial bioagent identifying amplicon abundance and calibration amplicon abundance indicates the quantity of bacterium in the sample. In some embodiments, the base composition of the bacterial bioagent identifying amplicon is determined.

[200] In some embodiments, the present invention provides methods for detecting or quantifying bacteria by combining a nucleic acid amplification process with a mass determination process. In some embodiments, such methods identify or otherwise analyze the bacterium by comparing mass information from an amplification product with a calibration or control product. Such methods can be carried out in a highly multiplexed and/or parallel manner allowing for the analysis of as many as 300 samples per 24 hours on a single mass measurement platform. The accuracy of the mass determination methods in some embodiments of the present invention permits allows for the ability to discriminate between different bacteria such as, for example, various genotypes and drug resistant strains of Staphylococcus aureus.

BRIEF DESCRIPTION OF THE DRAWINGS

[201] The foregoing summary of the invention, as well as the following detailed description of the invention, is better understood when read in conjunction with the accompanying drawings which are included by way of example and not by way of limitation.

- [202] Figure 1: process diagram illustrating a representative primer pair selection process.
- [203] Figure 2: process diagram illustrating an embodiment of the calibration method.
- [204] Figure 3: common pathogenic bacteria and primer pair coverage. The primer pair number in the upper right hand corner of each polygon indicates that the primer pair can produce a bioagent identifying amplicon for all species within that polygon.
- [205] Figure 4: a representative 3D diagram of base composition (axes A, G and C) of bioagent identifying amplicons obtained with primer pair number 14 (a precursor of primer pair number 348 which targets 16S rRNA). The diagram indicates that the experimentally determined base compositions of the clinical samples (labeled NHRC samples) closely match the base compositions expected for Sireptococcus pyogenes and are distinct from the expected base compositions of other organisms.
- [206] Figure 5: a representative mass spectrum of amplification products indicating the presence of bioagent identifying amplicons of Streptococcus pyogenes, Nelsseria meningitidis, and Haemophilus influenzae obtained from amplification of nucleic acid from a clinical sample with primer pair number 349 which targets 23S rRNA. Experimentally determined molecular masses and base compositions for the sense strand of each amplification product are shown.
- [207] Figure 6: a representative mass spectrum of amplification products representing a bioagent identifying amplicon of Streptococcus pyogenes, and a calibration amplicon obtained from amplification of nucleic acid from a clinical sample with primer pair number 356 which targets rplB. The experimentally determined molecular mass and base composition for the sense strand of the Streptococcus pyogenes amplification product is shown.
- [208] Figure 7: a representative mass spectrum of an amplified nucleic acid mixture which contained the Ames strain of *Bacillus anthracis*, a known quantity of combination calibration polynucleotide (SEO ID NO: 1464), and primer pair number 350 which targets the capC gene on

the virulence plasmid pX02 of *Bacillus anthracis*. Calibration amplicons produced in the amplification reaction are visible in the mass spectrum as indicated and abundance data (peak height) are used to calculate the quantity of the Ames strain of *Bacillus anthracis*.

DEFINITIONS

[209] As used herein, the term "abundance" refers to an amount. The amount may be described in terms of concentration which are common in molecular biology such as "copy number," "pfu or plate-forming unit" which are well known to those with ordinary skill. Concentration may be relative to a known standard or may be absolute,

[210] As used herein, the term "amplifiable nucleic acid" is used in reference to nucleic acids that may be amplified by any amplification method. It is contemplated that "amplifiable nucleic acid" also comprises "sample template."

[211] As used herein the term "amplification" refers to a special case of nucleic acid replication involving template specificity. It is to be contrasted with non-specific template replication (i.e., replication that is template-dependent but not dependent on a specific template). Template specificity is here distinguished from fidelity of replication (i.e., synthesis of the proper polynucleotide sequence) and nucleotide (ribo- or deoxyribo-) specificity. Template specificity is frequently described in terms of "target" specificity. Target sequences are "targets" in the sense that they are sought to be sorted out from other nucleic acid. Amplification techniques have been designed primarily for this sorting out. Template specificity is achieved in most amplification techniques by the choice of enzyme. Amplification enzymes are enzymes that, under conditions they are used, will process only specific sequences of nucleic acid in a heterogeneous mixture of nucleic acid. For example, in the case of QB replicase, MDV-1 RNA is the specific template for the replicase (D.L. Kacian et al., Proc. Natl. Acad. Sci. USA 69:3038 [1972]). Other nucleic acid will not be replicated by this amplification enzyme. Similarly, in the case of T7 RNA polymerase, this amplification enzyme has a stringent specificity for its own promoters (Chamberlin et al., Nature 228:227 [1970]). In the case of T4 DNA ligase, the enzyme will not ligate the two oligonucleotides or polynucleotides, where there is a mismatch between the oligonucleotide or polynucleotide substrate and the template at the ligation junction (D.Y. Wu and R. B. Wallace, Genomics 4:560 [1989]). Finally, Taq and Pfu polymerases, by virtue of their ability to function at high temperature, are found to display high specificity for the sequences bounded and thus defined by the primers; the high temperature results in thermodynamic conditions that favor primer hybridization with the target sequences and not hybridization with non-target sequences (H.A. Erlich (ed.), PCR Technology, Stockton Press [1989]).

[212] As used herein, the term "amplification reagents" refers to those reagents (deoxyribonucleotide triphosphates, buffer, etc.), needed for amplification, excluding primers, nucleic acid template, and the amplification enzyme. Typically, amplification reagents along with other reaction components are placed and contained in a reaction vessel (test tube, microwell, etc.).

- [213] As used herein, the term "analogous" when used in context of comparison of bioagent identifying amplicons indicates that the bioagent identifying amplicons being compared are produced with the same pair of primers. For example, bioagent identifying amplicon "A" and bioagent identifying amplicon "B", produced with the same pair of primers are analogous with respect to each other. Bioagent identifying amplicon "C", produced with a different pair of primers is not analogous to either bioagent identifying amplicon "A" or bioagent identifying amplicon "A" or bioagent identifying amplicon "B".
- [214] As used herein, the term "anion exchange functional group" refers to a positively charged functional group capable of binding an anion through an electrostatic interaction. The most well known anion exchange functional groups are the amines, including primary, secondary, tertiary and quaternary amines.
- [215] The term "bacteria" or "bacterium" refers to any member of the groups of eubacteria and archaebacteria
- [216] As used herein, a "base composition" is the exact number of each nucleobase (for example, A, T, C and G) in a segment of nucleic acid. For example, amplification of nucleic acid of Staphylococcus aureus strain carrying the lukS-PV gene with primer pair number 2095 (SEQ ID NOS: 456:1261) produces an amplification product 117 nucleobases in length from nucleic acid of the lukS-PV gene that has a base composition of A35 G17 C19 T46 (by convention with reference to the sense strand of the amplification product). Because the molecular masses of each of the four natural nucleotides and chemical modifications thereof are known (if applicable), a measured molecular mass can be deconvoluted to a list of possible base compositions. Identification of a base composition of a sense strand which is complementary to the corresponding antisense strand in terms of base composition provides a confirmation of the true base composition of an unknown amplification product. For example, the base composition of the antisense strand of the 139 nucleobase amplification product described above is A46 G19 C17 T35.
- [217] As used herein, a "base composition probability cloud" is a representation of the diversity in base composition resulting from a variation in sequence that occurs among different isolates of a given

species. The "base composition probability cloud" represents the base composition constraints for each species and is typically visualized using a pseudo four-dimensional plot.

- [218] In the context of this invention, a "bioagent" is any organism, cell, or virus, living or dead, or a nucleic acid derived from such an organism, cell or virus. Examples of bioagents include, but are not limited, to cells, (including but not limited to human clinical samples, bacterial cells and other pathogens), viruses, fungi, protists, parasites, and pathogenicity markers (including but not limited to: pathogenicity islands, antibiotic resistance genes, virulence factors, toxin genes and other bioregulating compounds). Samples may be alive or dead or in a vegetative state (for example, vegetative bacteria or spores) and may be encapsulated or bioengineered. In the context of this invention, a "pathogen" is a bioagent which causes a disease or disorder.
- [219] As used herein, a "bioagent division" is defined as group of bioagents above the species level and includes but is not limited to, orders, families, classes, clades, genera or other such groupings of bioagents above the species level.
- [220] As used herein, the term "bioagent identifying amplicon" refers to a polynucleotide that is amplified from a bioagent in an amplification reaction and which 1) provides sufficient variability to distinguish among bioagents from whose nucleic acid the bioagent identifying amplicon is produced and 2) whose molecular mass is amenable to a rapid and convenient molecular mass determination modality such as mass spectrometry, for example.
- [221] As used herein, the term "biological product" refers to any product originating from an organism. Biological products are often products of processes of biotechnology. Examples of biological products include, but are not limited to: cultured cell lines, cellular components, antibodies, proteins and other cell-derived biomolecules, growth media, growth harvest fluids, natural products and biopharmaceutical products.
- [222] The terms "biowarfare agent" and "bioweapon" are synonymous and refer to a bacterium, virus, fungus or protozoan that could be deployed as a weapon to cause bodily harm to individuals. Military or terrorist groups may be implicated in deployment of biowarfare agents.
- [223] In context of this invention, the term "broad range survey primer pair" refers to a primer pair designed to produce bioagent identifying amplicons across different broad groupings of bioagents. For example, the ribosomal RNA-targeted primer pairs are broad range survey primer pairs which have the capability of producing bacterial bioagent identifying amplicons for essentially all known bacteria. With

respect to broad range primer pairs employed for identification of bacteria, a broad range survey primer pair for bacteria such as 16S rRNA primer pair number 346 (SEQ ID NOs: 202:1110) for example, will produce an bacterial bioagent identifying amplicon for essentially all known bacteria.

- [224] The term "calibration amplicon" refers to a nucleic acid segment representing an amplification product obtained by amplification of a calibration sequence with a pair of primers designed to produce a bioagent identifying amplicon.
- [225] The term "calibration sequence" refers to a polynucle otide sequence to which a given pair of primers hybridizes for the purpose of producing an internal (i.e: included in the reaction) calibration standard amplification product for use in determining the quantity of a bioagent in a sample. The calibration sequence may be expressly added to an amplification reaction, or may already be present in the sample prior to analysis.
- [226] The term "clade primer pair" refers to a primer pair designed to produce bioagent identifying amplicons for species belonging to a clade group. A clade primer pair may also be considered as a "speciating" primer pair which is useful for distinguishing among closely related species.
- [227] The term "codon" refers to a set of three adjoined nucleotides (triplet) that codes for an amino acid or a termination signal.
- [228] In context of this invention, the term "codon base composition analysis," refers to determination of the base composition of an individual codon by obtaining a bioagent identifying amplicon that includes the codon. The bioagent identifying amplicon will at least include regions of the target nucleic acid sequence to which the primers hybridize for generation of the bioagent identifying amplicon as well as the codon being analyzed, located between the two primer hybridization regions.
- [229] As used herein, the terms "complementary" or "complementarity" are used in reference to polynucleotides (i.e., a sequence of nucleotides such as an oligonucleotide or a target nucleic acid) related by the base-pairing rules. For example, for the sequence "5'-A-G-T-3'," is complementary to the sequence "3'-T-C-A-5'." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids. Either term may also be used in reference to individual nucleotides, essecially within the

context of polynucleotides. For example, a particular nucleotide within an oligonucleotide may be noted for its complementarity, or lack thereof, to a nucleotide within another nucleic acid strand, in contrast or comparison to the complementarity between the rest of the oligonucleotide and the nucleic acid strand.

[230] The term "complement of a nucleic acid sequence" as used herein refers to an oligonucleotide which, when aligned with the nucleic acid sequence such that the 5' end of one sequence is paired with the 3' end of the other, is in "antiparallel association." Certain bases not commonly found in natural nucleic acids may be included in the nucleic acids of the present invention and include, for example, inosine and 7-deazaguanine. Complementarity need not be perfect; stable duplexes may contain mismatched base pairs or unmatched bases. Those skilled in the art of nucleic acid technology can determine duplex stability empirically considering a number of variables including, for example, the length of the oligonucleotide, base composition and sequence of the oligonucleotide, ionic strength and incidence of mismatched base pairs. Where a first oligonucleotide is complementary to a region of a target nucleic acid and a second oligonucleotide has complementary to the same region (or a portion of this region) a "region of overlap" exists along the target nucleic acid. The degree of overlap will vary depending upon the extent of the complementarity.

[231] In context of this invention, the term "division-wide primer pair" refers to a primer pair designed to produce bioagent identifying amplicons within sections of a broader spectrum of bioagents For example, primer pair number 352 (SEQ ID NOs: 687:1411), a division-wide primer pair, is designed to produce bacterial bioagent identifying amplicons for members of the Bacillus group of bacteria which comprises, for example, members of the genera Streptococci, Enterococci, and Staphylococci. Other division-wide primer pairs may be used to produce bacterial bioagent identifying amplicons for other groups of bacterial bioagents.

[232] As used herein, the term "concurrently amplifying" used with respect to more than one amplification reaction refers to the act of simultaneously amplifying more than one nucleic acid in a single reaction mixture.

[233] As used herein, the term "drill-down primer pair" refers to a primer pair designed to produce bioagent identifying amplicons for identification of sub-species characteristics or confirmation of a species assignment. For example, primer pair number 2146 (SEQ ID NOs. 437:1137), a drill-down Staphylococcus aureus genotyping primer pair, is designed to produce Staphylococcus aureus genotyping amplicons. Other drill-down primer pairs may be used to produce bioagent identifying amplicons for Staphylococcus aureus and other bacterial species.

[234] The term "duplex" refers to the state of nucleic acids in which the base portions of the nucleotides on one strand are bound through hydrogen bonding the their complementary bases arrayed on a second strand. The condition of being in a duplex form reflects on the state of the bases of a nucleic acid. By virtue of base pairing, the strands of nucleic acid also generally assume the tertiary structure of a double helix, having a major and a minor groove. The assumption of the helical form is implicit in the act of becoming duplexed.

- [235] As used herein, the term "etiology" refers to the causes or origins, of diseases or abnormal physiological conditions.
- [236] The term "gene" refers to a DNA sequence that comprises control and coding sequences necessary for the production of an RNA having a non-coding function (e.g., a ribosomal or transfer RNA), a polypeptide or a precursor. The RNA or polypeptide can be encoded by a full length coding sequence or by any portion of the coding sequence so long as the desired activity or function is retained.
- The terms "homology," "homologous" and "sequence identity" refer to a degree of identity. [237] There may be partial homology or complete homology. A partially homologous sequence is one that is less than 100% identical to another sequence. Determination of sequence identity is described in the following example: a primer 20 nucleobases in length which is otherwise identical to another 20 nucleobase primer but having two non-identical residues has 18 of 20 identical residues (18/20 = 0.9 or 90% sequence identity). In another example, a primer 15 nucleobases in length having all residues identical to a 15 nucleobase segment of a primer 20 nucleobases in length would have 15/20 = 0.75 or 75% sequence identity with the 20 nucleobase primer. In context of the present invention, sequence identity is meant to be properly determined when the query sequence and the subject sequence are both described and aligned in the 5' to 3' direction. Sequence alignment algorithms such as BLAST, will return results in two different alignment orientations. In the Plus/Plus orientation, both the query sequence and the subject sequence are aligned in the 5' to 3' direction. On the other hand, in the Plus/Minus orientation, the query sequence is in the 5' to 3' direction while the subject sequence is in the 3' to 5' direction. It should be understood that with respect to the primers of the present invention, sequence identity is properly determined when the alignment is designated as Plus/Plus. Sequence identity may also encompass alternate or modified nucleobases that perform in a functionally similar manner to the regular nucleobases adenine, thymine, guanine and cytosine with respect to hybridization and primer extension in amplification reactions. In a non-limiting example, if the 5-propynyl pyrimidines propyne C and/or propyne T replace one or more C or T residues in one primer which is otherwise identical to another primer in sequence and length, the two primers will have 100% sequence identity with each other. In another non-limiting example, Inosine (I) may be used as a replacement for G or T and effectively

hybridize to C, A or U (uracil). Thus, if inosine replaces one or more C, A or U residues in one primer which is otherwise identical to another primer in sequence and length, the two primers will have 100% sequence identity with each other. Other such modified or universal bases may exist which would perform in a functionally similar manner for hybridization and amplification reactions and will be understood to fall within this definition of sequence identity.

- [238] As used herein, "housekeeping gene" refers to a gene encoding a protein or RNA involved in basic functions required for survival and reproduction of a bioagent. Housekeeping genes include, but are not limited to genes encoding RNA or proteins involved in translation, replication, recombination and repair, transcription, nucleotide metabolism, amino acid metabolism, lipid metabolism, energy generation, uptake, secretion and the like.
- [239] As used herein, the term "hybridization" is used in reference to the pairing of complementary nucleic acids. Hybridization and the strength of hybridization (i.e., the strength of the association between the nucleic acids) is influenced by such factors as the degree of complementary between the nucleic acids, stringency of the conditions involved, and the T_m of the formed hybrid. "Hybridization" methods involve the annealing of one nucleic acid to another, complementary nucleic acid, i.e., a nucleic acid having a complementary nucleotide sequence. The ability of two polymers of nucleic acid containing complementary sequences to find each other and anneal through base pairing interaction is a well-recognized phenomenon. The initial observations of the "hybridization" process by Marmur and Lane, Proc. Natl. Acad. Sci. USA 46:461 (1960) have been followed by the refinement of this process into an essential tool of modem biology.
- [240] The term "in silico" refers to processes taking place via computer calculations. For example, electronic PCR (ePCR) is a process analogous to ordinary PCR except that it is carried out using nucleic acid sequences and primer pair sequences stored on a computer formatted medium.
- [241] As used herein, "intelligent primers" are primers that are designed to bind to highly conserved sequence regions of a bioagent identifying amplicon that flank an intervening variable region and, upon amplification, yield amplification products which ideally provide enough variability to distinguish individual bioagents, and which are amenable to molecular mass analysis. By the term "highly conserved," it is meant that the sequence regions exhibit between about 80-100%, or between about 95-100% identity among all, or at least 70%, at least 80%, at least 90%, at least 95%, or at least 90% of species or strains.

1242] The "ligase chain reaction" (LCR; sometimes referred to as "Ligase Amplification Reaction" (LAR) described by Barany, Proc. Natl. Acad. Sci., 88:189 (1991); Barany, PCR Methods and Applic., 1:5 (1991); and Wu and Wallace, Genomics 4:560 (1989) has developed into a well-recognized alternative method for amplifying nucleic acids. In LCR, four oligonucleotides, two adjacent oligonucleotides which uniquely hybridize to one strand of target DNA, and a complementary set of adjacent oligonucleotides, that hybridize to the opposite strand are mixed and DNA ligase is added to the mixture. Provided that there is complete complementarity at the junction, ligase will covalently link each set of hybridized molecules. Importantly, in LCR, two probes are ligated together only when they basepair with sequences in the target sample, without gaps or mismatches. Repeated cycles of denaturation, hybridization and ligation amplify a short segment of DNA. LCR has also been used in combination with PCR to achieve enhanced detection of single-base changes. However, because the four oligonucleotides used in this assay can pair to form two short ligatable fragments, there is the potential for the generation of target-independent background signal. The use of LCR for mutant screening is limited to the examination of specific nucleic acid positions.

- [243] The term "locked nucleic acid" or "LNA" refers to a nucleic acid analogue containing one or more 2'-O, 4'-C-methylene-\$\theta\$-D-ribofuranosyl nucleotide monomers in an RNA mimicking sugar conformation. LNA oligonucleotides display unprecedented hybridization affinity toward complementary single-stranded RNA and complementary single- or double-stranded DNA. LNA oligonucleotides induce A-type (RNA-like) duplex conformations. The primers of the present invention may contain LNA modifications.
- [244] As used herein, the term "mass-modifying tag" refers to any modification to a given nucleotide which results in an increase in mass relative to the analogous non-mass modified nucleotide. Mass-modifying tags can include heavy isotopes of one or more elements included in the nucleotide such as carbon-13 for example. Other possible modifications include addition of substituents such as iodine or bromine at the 5 position of the nucleobase for example.
- [245] The term "mass spectrometry" refers to measurement of the mass of atoms or molecules. The molecules are first converted to ions, which are separated using electric or magnetic fields according to the ratio of their mass to electric charge. The measured masses are used to identity the molecules.
- [246] The term "microorganism" as used herein means an organism too small to be observed with the unaided eye and includes, but is not limited to bacteria, virus, protozoans, fungi; and ciliates.

[247] The term "multi-drug resistant" or multiple-drug resistant" refers to a microorganism which is resistant to more than one of the antibiotics or antimicrobial agents used in the treatment of said microorganism.

- [248] The term "multiplex PCR" refers to a PCR reaction where more than one primer set is included in the reaction pool allowing 2 or more different DNA targets to be amplified by PCR in a single reaction tube.
- [249] The term "non-template tag" refers to a stretch of at least three guanine or cytosine nucleobases of a primer used to produce a bioagent identifying amplicon which are not complementary to the template. A non-template tag is incorporated into a primer for the purpose of increasing the primer-duplex stability of later cycles of amplification by incorporation of extra G-C pairs which each have one additional hydrogen bond relative to an A-T pair.
- [250] The term "nucleic acid sequence" as used herein refers to the linear composition of the nucleic acid residues A, T, C or G or any modifications thereof, within an oligonucleotide, nucleotide or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be single or double stranded, and represent the sense or antisense strand
- [251] As used herein, the term "nucleobase" is synonymous with other terms in use in the art including "nucleotide," "deoxynucleotide," "nucleotide residue," "deoxynucleotide residue," "nucleotide triphosphate (NTP)," or deoxynucleotide triphosphate (dNTP).
- [252] The term "nucleotide analog" as used herein refers to modified or non-naturally occurring nucleotides such as 5-propynyl pyrimidines (i.e., 5-propynyl-dTTP and 5-propynyl-dTCP), 7-deaza purines (i.e., 7-deaza-dATP and 7-deaza-dGTP). Nucleotide analogs include base analogs and comprise modified forms of deoxyribonucleotides as well as ribonucleotides.
- [253] The term "oligonucleotide" as used herein is defined as a molecule comprising two or more deoxyribonucleotides or ribonucleotides, preferably at least 5 nucleotides, more preferably at least about 13 to 35 nucleotides. The exact size will depend on many factors, which in turn depend on the ultimate function or use of the oligonucleotide. The oligonucleotide may be generated in any manner, including chemical synthesis, DNA replication, reverse transcription, PCR, or a combination thereof. Because mononucleotides are reacted to make oligonucleotides in a manner such that the 5' phosphate of one mononucleotide pentose ring is attached to the 3' oxygen of its neighbor in one direction via a phosphodiester linkage, an end of an oligonucleotide is referred to as the "5'-end" if its 5' phosphate is not

linked to the 3' oxygen of a mononucleotide pentose ring and as the "3'-end" if its 3' oxygen is not linked to a 5' phosphate of a subsequent mononucleotide pentose ring. As used herein, a nucleic acid sequence, even if internal to a larger oligonucleotide, also may be said to have 5' and 3' ends. A first region along a nucleic acid strand is said to be upstream of another region if the 3' end of the first region is before the 5' end of the second region when moving along a strand of nucleic acid in a 5' to 3' direction. All oligonucleotide primers disclosed herein are understood to be presented in the 5' to 3' direction when reading left to right. When two different, non-overlapping oligonucleotides anneal to different regions of the same linear complementary nucleic acid sequence, and the 3' end of one oligonucleotide points towards the 5' end of the other, the former may be called the "upstream" oligonucleotide and the latter the "downstream" oligonucleotide. Similarly, when two overlapping oligonucleotides are hybridized to the same linear complementary nucleic acid sequence, with the first oligonucleotide positioned such that its 5' end is upstream of the 5' end of the second oligonucleotide, and the 3' end of the first oligonucleotide is upstream of the 5' end of the second oligonucleotide, the first oligonucleotide may be called the "upstream" oligonucleotide and the second oligonucleotide may be called the "downstream" oligonucleotide.

- [254] In the context of this invention, a "pathogen" is a bioagent which causes a disease or disorder.
- [255] As used herein, the terms "PCR product," "PCR fragment," and "amplification product" refer to the resultant mixture of compounds after two or more cycles of the PCR steps of denaturation, annealing and extension are complete. These terms encompass the case where there has been amplification of one or more segments of one or more target sequences.
- [256] The term "peptide nucleic acid" ("PNA") as used herein refers to a molecule comprising bases or base analogs such as would be found in natural nucleic acid, but attached to a peptide backbone rather than the sugar-phosphate backbone typical of nucleic acids. The attachment of the bases to the peptide is such as to allow the bases to base pair with complementary bases of nucleic acid in a manner similar to that of an oligonucleotide. These small molecules, also designated anti gene agents, stop transcript elongation by binding to their complementary strand of nucleic acid (Nielsen, et al. Anticancer Drug Des. 8:53 63). The primers of the present invention may comprise PNAs.
- [257] The term "polymerase" refers to an enzyme having the ability to synthesize a complementary strand of nucleic acid from a starting template nucleic acid strand and free dNTPs.
- [258] As used herein, the term "polymerase chain reaction" ("PCR") refers to the method of K.B. Mullis U.S. Patent Nos. 4,683,195, 4,683,202, and 4,965,188, hereby incorporated by reference, that

describe a method for increasing the concentration of a segment of a target sequence in a mixture of genomic DNA without cloning or purification. This process for amplifying the target sequence consists of introducing a large excess of two oligonucleotide primers to the DNA mixture containing the desired target sequence, followed by a precise sequence of thermal cycling in the presence of a DNA polymerase. The two primers are complementary to their respective strands of the double stranded target sequence. To effect amplification, the mixture is denatured and the primers then annealed to their complementary sequences within the target molecule. Following annealing, the primers are extended with a polymerase so as to form a new pair of complementary strands. The steps of denaturation, primer annealing, and polymerase extension can be repeated many times (i.e., denaturation, annealing and extension constitute one "cycle"; there can be numerous "cycles") to obtain a high concentration of an amplified segment of the desired target sequence. The length of the amplified segment of the desired target sequence is determined by the relative positions of the primers with respect to each other, and therefore, this length is a controllable parameter. By virtue of the repeating aspect of the process, the method is referred to as the "polymerase chain reaction" (hereinafter "PCR"). Because the desired amplified segments of the target sequence become the predominant sequences (in terms of concentration) in the mixture, they are said to be "PCR amplified." With PCR, it is possible to amplify a single copy of a specific target sequence in genomic DNA to a level detectable by several different methodologies (e.g., hybridization with a labeled probe; incorporation of biotinylated primers followed by avidin-enzyme conjugate detection; incorporation of 32P-labeled deoxynucleotide triphosphates, such as dCTP or dATP, into the amplified segment). In addition to genomic DNA, any oligonucleotide or polynucleotide sequence can be amplified with the appropriate set of primer molecules. In particular, the amplified segments created by the PCR process itself are, themselves, efficient templates for subsequent PCR amplifications.

[259] The term "polymerization means" or "polymerization agent" refers to any agent capable of facilitating the addition of nucleoside triphosphates to an oligonucleotide. Preferred polymerization means comprise DNA and RNA polymerases.

[260] As used herein, the terms "pair of primers," or "primer pair" are synonymous. A primer pair is used for amplification of a nucleic acid sequence. A pair of primers comprises a forward primer and a reverse primer. The forward primer hybridizes to a sense strand of a target gene sequence to be amplified and primes synthesis of an antisense strand (complementary to the sense strand) using the target sequence as a template. A reverse primer hybridizes to the antisense strand of a target gene sequence to be amplified and primes synthesis of a sense strand (complementary to the antisense strand) using the target sequence as a template.

[261] The primers are designed to bind to highly conserved sequence regions of a bioagent identifying amplicon that flank an intervening variable region and yield amplification products which ideally provide enough variability to distinguish each individual bioagent, and which are amenable to molecular mass analysis. In some embodiments, the highly conserved sequence regions exhibit between about 80-100%, or between about 90-100%, or between about 95-100% identity, or between about 99-100% identity. The molecular mass of a given amplification product provides a means of identifying the bioagent from which it was obtained, due to the variability of the variable region. Thus design of the primers requires selection of a variable region with appropriate variability to resolve the identity of a given bioagent. Bioagent identifying amplicons are ideally specific to the identity of the bioagent.

[262] Properties of the primers may include any number of properties related to structure including, but not limited to: nucleobase length which may be contiguous (linked together) or non-contiguous (for example, two or more contiguous segments which are joined by a linker or loop moiety), modified or universal nucleobases (used for specific purposes such as for example, increasing hybridization affinity, preventing non-templated adenylation and modifying molecular mass) percent complementarity to a given target sequences.

[263] Properties of the primers also include functional features including, but not limited to, orientation of hybridization (forward or reverse) relative to a nucleic acid template. The coding or sense strand is the strand to which the forward priming primer hybridizes (forward priming orientation) while the reverse priming primer hybridizes to the non-coding or antisense strand (reverse priming orientation). The functional properties of a given primer pair also include the generic template nucleic acid to which the primer pair hybridizes. For example, identification of bioagents can be accomplished at different levels using primers suited to resolution of each individual level of identification. Broad range survey primers are designed with the objective of identifying a bioagent as a member of a particular division (e.g., an order, family, genus or other such grouping of bioagents above the species level of bioagents). In some embodiments, broad range survey intelligent primers are capable of identification of bioagents at the species or sub-species level. Other primers may have the functionality of producing bioagent identifying amplicons for members of a given taxonomic genus, clade, species, sub-species or genotype (including genetic variants which may include presence of virulence genes or antibiotic resistance genes or mutations). Additional functional properties of primer pairs include the functionality of performing amplification either singly (single primer pair per amplification reaction vessel) or in a multiplex fashion (multiple primer pairs and multiple amplification reactions within a single reaction vessel).

[264] As used herein, the terms "purified" or "substantially purified" refer to molecules, either nucleic or amino acid sequences, that are removed from their natural environment, isolated or separated,

and are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which they are naturally associated. An "isolated polynucleotide" or "isolated oligonucleotide" is therefore a substantially purified polynucleotide.

[265] The term "reverse transcriptase" refers to an enzyme having the ability to transcribe DNA from an RNA template. This enzymatic activity is known as reverse transcriptase activity. Reverse transcriptase activity is desirable in order to obtain DNA from RNA viruses which can then be amplified and analyzed by the methods of the present invention.

[266] The term "ribosomal RNA" or "rRNA" refers to the primary ribonucleic acid constituent of ribosomes. Ribosomes are the protein-manufacturing organelles of cells and exist in the cytoplasm. Ribosomal RNAs are transcribed from the DNA genes encoding them.

1267] The term "sample" in the present specification and claims is used in its broadest sense. On the one hand it is meant to include a specimen or culture (e.g., microbiological cultures). On the other hand, it is meant to include both biological and environmental samples. A sample may include a specimen of synthetic origin. Biological samples may be animal, including human, fluid, solid (e.g., stool) or tissue, as well as liquid and solid food and feed products and ingredients such as dairy items, vegetables, meat and meat by-products, and waste. Biological samples may be obtained from all of the various families of domestic animals, as well as feral or wild animals, including, but not limited to, such animals as ungulates, bear, fish, lagamorphs, rodents, etc. Environmental samples include environmental material such as surface matter, soil, water, air and industrial samples, as well as samples obtained from food and dairy processing instruments, apparatus, equipment, utensils, disposable and non-disposable items. These examples are not to be construed as limiting the sample types applicable to the present invention. The term "source of target nucleic acid" refers to any sample that contains nucleic acids (RNA or DNA). Particularly preferred sources of target nucleic acids are biological samples including, but not limited to blood, saliva, cerebral spinal fluid, pleural fluid, milk, lymph, sputum and semen.

[268] As used herein, the term "sample template" refers to nucleic acid originating from a sample that is analyzed for the presence of "target" (defined below). In contrast, "background template" is used in reference to nucleic acid other than sample template that may or may not be present in a sample. Background template is often a contaminant. It may be the result of carryover, or it may be due to the presence of nucleic acid contaminants sought to be purified away from the sample. For example, nucleic acids from organisms other than those to be detected may be present as background in a test sample.

[269] A "segment" is defined herein as a region of nucleic acid within a target sequence.

The "self-sustained sequence replication reaction" (3RR) (Guatelli et al., Proc. Natl. Acad. Sci., 87:1874-1878 [1990], with an erratum at Proc. Natl. Acad. Sci., 87:1777 [1990]) is a transcription-based in vitro amplification system (Kwok et al., Proc. Natl. Acad. Sci., 86:1173-1177 [1980]) that can exponentially amplify RNA sequences at a uniform temperature. The amplified RNA can then be utilized for mutation detection (Fahy et al., PCR Meth. Appl., 1:25-33 [1991]). In this method, an oligonucleotide primer is used to add a phage RNA polymerase promoter to the 5' end of the sequence of interest. In a cocktail of enzymes and substrates that includes a second primer, reverse transcriptase, RNase H, RNA polymerase and ribo- and deoxyribonucleoside triphosphates, the target sequence undergoes repeated rounds of transcription, cDNA synthesis and second-strand synthesis to amplify the area of interest. The use of 35R to detect mutations is kinetically limited to screening small segments of DNA (e.g., 200-300 base pairs).

[271] As used herein, the term ""sequence alignment!"" refers to a listing of multiple DNA or amino acid sequences and aligns them to highlight their similarities. The listings can be made using bioinformatics computer programs.

[272] In context of this invention, the term "speciating primer pair" refers to a primer pair designed to produce a bioagent identifying amplicon with the diagnostic capability of identifying species members of a group of genera or a particular genus of bioagents. Primer pair number 2249 (SEQ ID NOs: 430:1321), for example, is a speciating primer pair used to distinguish Staphylococcus aureus from other species of the genus Staphylococcus.

273] As used herein, a "sub-species characteristic" is a genetic characteristic that provides the means to distinguish two members of the same bioagent species. For example, one viral strain could be distinguished from another viral strain of the same species by possessing a genetic change (e.g., for example, a nucleotide deletion, addition or substitution) in one of the viral genes, such as the RNA-dependent RNA polymerase. Sub-species characteristics such as virulence genes and drug-are responsible for the phenotypic differences among the different strains of bacteria.

As used herein, the term "target" is used in a broad sense to indicate the gene or genomic region being amplified by the primers. Because the present invention provides a plurality of amplification products from any given primer pair (depending on the bioagent being analyzed), multiple amplification products from different specific nucleic acid sequences may be obtained. Thus, the term "target" is not used to refer to a single specific nucleic acid sequence. The "target" is sought to be sorted out from other nucleic acid sequences and contains a sequence that has at least partial complementarity with an

oligonucleotide primer. The target nucleic acid may comprise single- or double-stranded DNA or RNA. A "segment" is defined as a region of nucleic acid within the target sequence.

- [275] The term "template" refers to a strand of nucleic acid on which a complementary copy is built from nucleoside triphosphates through the activity of a template-dependent nucleic acid polymerase.

 Within a duplex the template strand is, by convention, depicted and described as the "bottom" strand.

 Similarly, the non-template strand is often depicted and described as the "top" strand.
- [276] As used herein, the term "T_m" is used in reference to the "melting temperature." The melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. Several equations for calculating the T_m of nucleic acids are well known in the art. As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation: T_m=81.5+0.41(% G+C), when a nucleic acid is in aqueous solution at 1 M NaC1 (see e.g., Anderson and Young, Quantitative Filter Hybridization, in Nucleic Acid Hybridization (1985). Other references (e.g., Allawi, H. T. & SantaLucia, J., Jr. Thermodynamics and NMR of internal G.T mismatches in DNA. Biochemistry 36, 10581-94 (1997) include more sophisticated computations which take structural and environmental, as well as sequence characteristics into account for the calculation of T_m.
- [277] The term "triangulation genotyping analysis" refers to a method of genotyping a bioagent by measurement of molecular masses or base compositions of amplification products, corresponding to bioagent identifying amplicons, obtained by amplification of regions of more than one gene. In this sense, the term "triangulation" refers to a method of establishing the accuracy of information by comparing three or more types of independent points of view bearing on the same findings. Triangulation genotyping analysis carried out with a plurality of triangulation genotyping analysis primers yields a plurality of base compositions that then provide a pattern or "barcode" from which a species type can be assigned. The species type may represent a previously known sub-species or strain, or may be a previously unknown strain having a specific and previously unobserved base composition barcode indicating the existence of a previously unknown genotype.
- [278] As used herein, the term "triangulation genotyping analysis primer pair" is a primer pair designed to produce bioagent identifying amplicons for determining species types in a triangulation genotyping analysis.
- [279] The employment of more than one bioagent identifying amplicon for identification of a bioagent is herein referred to as "triangulation identification." Triangulation identification is pursued by

analyzing a plurality of bioagent identifying amplicons produced with different primer pairs. This process is used to reduce false negative and false positive signals, and enable reconstruction of the origin of hybrid or otherwise engineered bioagents. For example, identification of the three part toxin genes typical of B. anthracis (Bowen et al., J. Appl. Microbiol., 1999, 87, 270-278) in the absence of the expected signatures from the B. anthracis genome would suggest a genetic engineering event.

In the context of this invention, the term "unknown bioagent" may mean either: (i) a bioagent whose existence is known (such as the well known bacterial species Staphylococcus aureus for example) but which is not known to be in a sample to be analyzed, or (ii) a bioagent whose existence is not known (for example, the SARS coronavirus was unknown prior to April 2003). For example, if the method for identification of coronaviruses disclosed in commonly owned U.S. Patent Serial No. 10/829,826 (incorporated herein by reference in its entirety) was to be employed prior to April 2003 to identify the SARS coronavirus in a clinical sample, both meanings of "unknown" bioagent are applicable since the SARS coronavirus was unknown to science prior to April, 2003 and since it was not known what bioagent (in this case a coronavirus) was present in the sample. On the other hand, if the method of U.S. Patent Serial No. 10/829,826 was to be employed subsequent to April 2003 to identify the SARS coronavirus in a clinical sample, only the first meaning (i) of "unknown" bioagent would apply since the SARS coronavirus became known to science subsequent to April 2003 and since it was not known what bioagent was present in the sample.

[281] The term "variable sequence" as used herein refers to differences in nucleic acid sequence between two nucleic acids. For example, the genes of two different bacterial species may vary in sequence by the presence of single base substitutions and/or deletions or insertions of one or more nucleotides. These two forms of the structural gene are said to vary in sequence from one another. In the context of the present invention, "viral nucleic acid" includes, but is not limited to, DNA, RNA, or DNA that has been obtained from viral RNA, such as, for example, by performing a reverse transcription reaction. Viral RNA can either be single-stranded (of positive or negative polarity) or double-stranded.

[282] The term "virus" refers to obligate, ultramicroscopic, parasites that are incapable of autonomous replication (i.e., replication requires the use of the host cell's machinery). Viruses can survive outside of a host cell but cannot replicate.

[283] The term "wild-type" refers to a gene or a gene product that has the characteristics of that gene or gene product when isolated from a naturally occurring source. A wild-type gene is that which is most frequently observed in a population and is thus arbitrarily designated the "normal" or "wild-type" form of the gene. In contrast, the term "modified", "mutant" or "polymorphic" refers to a gene or gene product

that displays modifications in sequence and or functional properties (i.e., altered characteristics) when compared to the wild-type gene or gene product. It is noted that naturally-occurring mutants can be isolated; these are identified by the fact that they have altered characteristics when compared to the wildtype gene or gene product.

[284] As used herein, a "wobble base" is a variation in a codon found at the third nucleotide position of a DNA triplet. Variations in conserved regions of sequence are often found at the third nucleotide position due to redundancy in the amino acid code.

DETAILED DESCRIPTION OF EMBODIMENTS

A. Bioagent Identifying Amplicons

The present invention provides methods for detection and identification of unknown bioagents [285] using bioagent identifying amplicons. Primers are selected to hybridize to conserved sequence regions of nucleic acids derived from a bioagent, and which bracket variable sequence regions to yield a bioagent identifying amplicon, which can be amplified and which is amenable to molecular mass determination. The molecular mass then provides a means to uniquely identify the bioagent without a requirement for prior knowledge of the possible identity of the bioagent. The molecular mass or corresponding base composition signature of the amplification product is then matched against a database of molecular masses or base composition signatures. A match is obtained when an experimentally-determined molecular mass or base composition of an analyzed amplification product is compared with known molecular masses or base compositions of known bioagent identifying amplicons and the experimentally determined molecular mass or base composition is the same as the molecular mass or base composition of one of the known bioagent identifying amplicons. Alternatively, the experimentally-determined molecular mass or base composition may be within experimental error of the molecular mass or base composition of a known bioagent identifying amplicon and still be classified as a match. In some cases, the match may also be classified using a probability of match model such as the models described in U.S. Serial No. 11/073,362, which is commonly owned and incorporated herein by reference in entirety. Furthermore, the method can be applied to rapid parallel multiplex analyses, the results of which can be employed in a triangulation identification strategy. The present method provides rapid throughput and does not require nucleic acid sequencing of the amplified target sequence for bioagent detection and identification.

[286] Despite enormous biological diversity, all forms of life on earth share sets of essential, common features in their genomes. Since genetic data provide the underlying basis for identification of bioagents by the methods of the present invention, it is necessary to select segments of nucleic acids which ideally provide enough variability to distinguish each individual bioagent and whose molecular mass is amenable to molecular mass determination.

[287] Unlike bacterial genomes, which exhibit conservation of numerous genes (i.e. housekeeping genes) across all organisms, viruses do not share a gene that is essential and conserved among all virus families. Therefore, viral identification is achieved within smaller groups of related viruses, such as members of a particular virus family or genus. For example, RNA-dependent RNA polymerase is present in all single-stranded RNA viruses and can be used for broad priming as well as resolution within the virus family.

[288] In some embodiments of the present invention, at least one bacterial nucleic acid segment is amplified in the process of identifying the bacterial bioagent. Thus, the nucleic acid segments that can be amplified by the primers disclosed herein and that provide enough variability to distinguish each individual bioagent and whose molecular masses are amenable to molecular mass determination are herein described as bioagent identifying amplicons.

[289] In some embodiments of the present invention, bioagent identifying amplicons comprise from about 45 to about 150 nucleobases (i.e. from about 45 to about 200 linked nucleosides), although both longer and short regions may be used. One of ordinary skill in the art will appreciate that the invention embodies compounds of 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, and 150 nucleobases in length, or any range therewithin.

[290] It is the combination of the portions of the bioagent nucleic acid segment to which the primers hybridize (hybridization sites) and the variable region between the primer hybridization sites that comprises the bioagent identifying amplicon. Thus, it can be said that a given bioagent identifying amplicon is "defined by" a given pair of primers.

[291] In some embodiments, bioagent identifying amplicons amenable to molecular mass determination which are produced by the primers described herein are either of a length, size or mass compatible with the particular mode of molecular mass determination or compatible with a means of providing a predictable fragmentation pattern in order to obtain predictable fragments of a length compatible with the particular mode of molecular mass determination. Such means of providing a predictable fragmentation pattern of an amplification product include, but are not limited to, cleavage with chemical reasents, restriction enzymes or cleavage primers, for example. Thus, in some

embodiments, bioagent identifying amplicons are larger than 150 nucleobases and are amenable to molecular mass determination following restriction digestion. Methods of using restriction enzymes and cleavage primers are well known to those with ordinary skill in the art.

[292] In some embodiments, amplification products corresponding to bioagent identifying amplicons are obtained using the polymerase chain reaction (PCR) that is a routine method to those with ordinary skill in the molecular biology arts. Other amplification methods may be used such as ligase chain reaction (LCR), low-stringency single primer PCR, and multiple strand displacement amplification (MDA). These methods are also known to those with ordinary skill.

B. Primers and Primer Pairs

[293] In some embodiments, the primers are designed to bind to conserved sequence regions of a bioagent identifying amplicon that flank an intervening variable region and yield amplification products which provide variability sufficient to distinguish each individual bioagent, and which are amenable to molecular mass analysis. In some embodiments, the highly conserved sequence regions exhibit between about 80-100%, or between about 90-100%, or between about 95-100% identity, or between about 99-100% identity. The molecular mass of a given amplification product provides a means of identifying the bioagent from which it was obtained, due to the variability of the variable region. Thus, design of the primers involves selection of a variable region with sufficient variability to resolve the identity of a given bioagent. In some embodiments, bioagent identifying amplicons are specific to the identity of the bioagent.

[294] In some embodiments, identification of bioagents is accomplished at different levels using primers suited to resolution of each individual level of identification. Broad range survey primers are designed with the objective of identifying a bioagent as a member of a particular division (e.g., an order, family, genus or other such grouping of bioagents above the species level of bioagents). In some embodiments, broad range survey intelligent primers are capable of identification of bioagents at the species or sub-species level. Examples of broad range survey primers include, but are not limited to: primer pair numbers: 346 (SEQ ID NOs: 202:1110), 347 (SEQ ID NOs: 560:1278), 348 SEQ ID NOs: 706:895), and 361 (SEQ ID NOs: 697:1398) which target DNA encoding 16S rRNA, and primer pair numbers 349 (SEQ ID NOs: 401:1156) and 360 (SEQ ID NOs: 409:1434) which target DNA encoding 23S rRNA.

[295] In some embodiments, drill-down primers are designed with the objective of identifying a bioagent at the sub-species level (including strains, subtypes, variants and isolates) based on sub-species characteristics which may, for example, include single nucleotide polymorphisms (SNPs), variable

number tandem repeats (VNTRs), deletions, drug resistance mutations or any other modification of a nucleic acid sequence of a bioagent relative to other members of a species having different sub-species characteristics. Drill-down intelligent primers are not always required for identification at the sub-species level because broad range survey intelligent primers may, in some cases provide sufficient identification resolution to accomplishing this identification objective. Examples of drill-down primers include, but are not limited to: confirmation primer pairs such as primer pair numbers 351 (SEQ ID NOs: 355:1423) and 353 (SEQ ID NOs: 220:1394), which target the pX01 virulence plasmid of Bacillus anthracis. Other examples of drill-down primer pairs are found in sets of triangulation genotyping primer pairs such as, for example, the primer pair number 2146 (SEQ ID NOs: 437:1137) which targets the arcC gene (encoding carmabate kinase) and is included in an 8 primer pair panel or kit for use in genotyping Staphylococcus aureus, or in other panels or kits of primer pairs used for determining drug-resistant bacterial strains, such as, for example, primer pair number 2095 (SEQ ID NOs: 456:1261) which targets the pv-luk gene (encoding Panton-Valentine leukocidin) and is included in an 8 primer pair panel or kit for use in identification of drug resistant strains of Staphylococcus aureus.

A representative process flow diagram used for primer selection and validation process is 12961 outlined in Figure 1. For each group of organisms, candidate target sequences are identified (200) from which nucleotide alignments are created (210) and analyzed (220). Primers are then designed by selecting appropriate priming regions (230) to facilitate the selection of candidate primer pairs (240). The primer pairs are then subjected to in silico analysis by electronic PCR (ePCR) (300) wherein bioagent identifying amplicons are obtained from sequence databases such as GenBank or other sequence collections (310) and checked for specificity in silico (320). Bioagent identifying amplicons obtained from GenBank sequences (310) can also be analyzed by a probability model which predicts the capability of a given amplicon to identify unknown bioagents such that the base compositions of amplicons with favorable probability scores are then stored in a base composition database (325). Alternatively, base compositions of the bioagent identifying amplicons obtained from the primers and GenBank sequences can be directly entered into the base composition database (330). Candidate primer pairs (240) are validated by testing their ability to hybridize to target nucleic acid by an in vitro amplification by a method such as PCR analysis (400) of nucleic acid from a collection of organisms (410). Amplification products thus obtained are analyzed by gel electrophoresis or by mass spectrometry to confirm the sensitivity, specificity and reproducibility of the primers used to obtain the amplification products (420).

[297] Many of the important pathogens, including the organisms of greatest concern as biowarfare agents, have been completely sequenced. This effort has greatly facilitated the design of primers for the detection of unknown bioagents. The combination of broad-range priming with division-wide and drill-down priming has been used very successfully in several applications of the technology, including

environmental surveillance for biowarfare threat agents and clinical sample analysis for medically important pathogens.

[298] Synthesis of primers is well known and routine in the art. The primers may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, CA). Any other means for such synthesis known in the art may additionally or alternatively be employed.

[299] In some embodiments primers are employed as compositions for use in methods for identification of bacterial bioagents as follows: a primer pair composition is contacted with nucleic acid (such as, for example, bacterial DNA or DNA reverse transcribed from the rRNA) of an unknown bacterial bioagent. The nucleic acid is then amplified by a nucleic acid amplification technique, such as PCR for example, to obtain an amplification product that represents a bioagent identifying amplicon. The molecular mass of each strand of the double-stranded amplification product is determined by a molecular mass measurement technique such as mass spectrometry for example, wherein the two strands of the double-stranded amplification product are separated during the ionization process. In some embodiments, the mass spectrometry is electrospray Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS) or electrospray time of flight mass spectrometry (ESI-TOF-MS). A list of possible base compositions can be generated for the molecular mass value obtained for each strand and the choice of the correct base composition from the list is facilitated by matching the base composition of one strand with a complementary base composition of the other strand. The molecular mass or base composition thus determined is then compared with a database of molecular masses or base compositions of analogous bioagent identifying amplicons for known viral bioagents. A match between the molecular mass or base composition of the amplification product and the molecular mass or base composition of an analogous bioagent identifying amplicon for a known viral bioagent indicates the identity of the unknown bioagent. In some embodiments, the primer pair used is one of the primer pairs of Table 2. In some embodiments, the method is repeated using one or more different primer pairs to resolve possible ambiguities in the identification process or to improve the confidence level for the identification assignment.

[300] In some embodiments, a bioagent identifying amplicon may be produced using only a single primer (either the forward or reverse primer of any given primer pair), provided an appropriate amplification method is chosen, such as, for example, low stringency single primer PCR (LSSP-PCR). Adaptation of this amplification method in order to produce bioagent identifying amplicons can be accomplished by one with ordinary skill in the art without undue experimentation.

[301] In some embodiments, the oligonucleotide primers are broad range survey primers which hybridize to conserved regions of nucleic acid encoding the hexon gene of all (or between 80% and 100%, between 85% and 100%, between 90% and 100% or between 95% and 100%) known bacteria and produce bacterial bioagent identifying amplicons.

[302] In some cases, the molecular mass or base composition of a bacterial bioagent identifying amplicon defined by a broad range survey primer pair does not provide enough resolution to unambiguously identify a bacterial bioagent at or below the species level. These cases benefit from further analysis of one or more bacterial bioagent identifying amplicons generated from at least one additional broad range survey primer pair or from at least one additional division-wide primer pair. The employment of more than one bioagent identifying amplicon for identification of a bioagent is herein referred to as triangulation identification.

[303] In other embodiments, the oligonucleotide primers are division-wide primers which hybridize to nucleic acid encoding genes of species within a genus of bacteria. In other embodiments, the oligonucleotide primers are drill-down primers which enable the identification of sub-species characteristics. Drill down primers provide the functionality of producing bioagent identifying amplicons for drill-down analyses such as strain typing when contacted with nucleic acid under amplification conditions. Identification of such sub-species characteristics is often critical for determining proper clinical treatment of viral infections. In some embodiments, sub-species characteristics are identified using only broad range survey primers and division-wide and drill-down primers are not used.

[304] In some embodiments, the primers used for amplification hybridize to and amplify genomic DNA, and DNA of bacterial plasmids.

In some embodiments, various computer software programs may be used to aid in design of primers for amplification reactions such as Primer Premier 5 (Premier Biosoft, Palo Alto, CA) or OLIGO Primer Analysis Software (Molecular Biology Insights, Cascade, CO). These programs allow the user to input desired hybridization conditions such as melting temperature of a primer-template duplex for example. In some embodiments, an in silico PCR search algorithm, such as (ePCR) is used to analyze primer specificity across a plurality of template sequences which can be readily obtained from public sequence databases such as GenBank for example. An existing RNA structure search algorithm (Macke et al., Nucl. Acids Res., 2001, 29, 4724-4735, which is incorporated herein by reference in its entirety) has been modified to include PCR parameters such as hybridization conditions, mismatches, and thermodynamic calculations (SantaLucia, Proc. Natl. Acad. Sci. U.S.A., 1998, 95, 1460-1465, which is incorporated herein by reference in its entirety).

the selected primer pairs. In some embodiments, the hybridization conditions applied to the algorithm can limit the results of primer specificity obtained from the algorithm. In some embodiments, the melting temperature threshold for the primer template duplex is specified to be 35°C or a higher temperature. In some embodiments the number of acceptable mismatches is specified to be seven mismatches or less. In some embodiments, the buffer components and concentrations and primer concentrations may be specified and incorporated into the algorithm, for example, an appropriate primer concentration is about 250 nM and appropriate buffer components are 50 mM sodium or potassium and 1.5 mM Mg²⁺.

One with ordinary skill in the art of design of amplification primers will recognize that a given primer need not hybridize with 100% complementarity in order to effectively prime the synthesis of a complementary nucleic acid strand in an amplification reaction. Moreover, a primer may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event. (e.g., for example, a loop structure or a hairpin structure). The primers of the present invention may comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 90% or at least 99% sequence identity with any of the primers listed in Table 2. Thus, in some embodiments of the present invention, an extent of variation of 70% to 100%, or any range therewithin, of the sequence identity is possible relative to the specific primer sequences disclosed herein. Determination of sequence identity is described in the following example: a primer 20 nucleobases in length which is identical to another 20 nucleobase primer having two non-identical residues has 18 of 20 identical residues (18/20 = 0.9 or 90% sequence identity). In another example, a primer 15 nucleobases in length having all residues identical to a 15 nucleobase segment of primer 20 nucleobases in length would have 15/20 = 0.75 or 75% sequence identity with the 20 nucleobase primer.

[307] Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for UNIX, Genetics Computer Group, University Research Park, Madison WI), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482-489). In some embodiments, complementarity of primers with respect to the conserved priming regions of viral nucleic acid is between about 70% and about 75% 80%. In other embodiments, homology, sequence identity or complementarity, is between about 75% and about 80%. In yet other embodiments, homology, sequence identity or complementarity, is at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 98%, at least 98%, at least 99% or is 100%.

[308] In some embodiments, the primers described herein comprise at least 70%, at least 75%, at least 80%, at least 80%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 98%,

or at least 99%, or 100% (or any range therewithin) sequence identity with the primer sequences specifically disclosed herein.

[309] One with ordinary skill is able to calculate percent sequence identity or percent sequence homology and able to determine, without undue experimentation, the effects of variation of primer sequence identity on the function of the primer in its role in priming synthesis of a complementary strand of nucleic acid for production of an amplification product of a corresponding bioagent identifying amplicon.

[310] In one embodiment, the primers are at least 13 nucleobases in length. In another embodiment, the primers are less than 36 nucleobases in length.

[311] In some embodiments of the present invention, the oligonucleotide primers are 13 to 35 nucleobases in length (13 to 35 linked nucleotide residues). These embodiments comprise oligonucleotide primers 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or 35 nucleobases in length, or any range therewithin. The present invention contemplates using both longer and shorter primers. Furthermore, the primers may also be linked to one or more other desired moieties, including, but not limited to, affinity groups, ligands, regions of nucleic acid that are not complementary to the nucleic acid to be amplified, labels, etc. Primers may also form hairpin structures. For example, hairpin primers may be used to amplify short target nucleic acid molecules. The presence of the hairpin may stabilize the amplification complex (see e.g., TAQMAN MicroRNA Assays, Applied Biosystems, Foster City, California).

[312] In some embodiments, any oligonucleotide primer pair may have one or both primers with less then 70% sequence homology with a corresponding member of any of the primer pairs of Table 2 if the primer pair has the capability of producing an amplification product corresponding to a bioagent identifying amplicon. In other embodiments, any oligonucleotide primer pair may have one or both primers with a length greater than 35 nucleobases if the primer pair has the capability of producing an amplification product corresponding to a bioagent identifying amplicon.

[313] In some embodiments, the function of a given primer may be substituted by a combination of two or more primers segments that hybridize adjacent to each other or that are linked by a nucleic acid loop structure or linker which allows a polymerase to extend the two or more primers in an amplification reaction.

In some embodiments, the primer pairs used for obtaining bioagent identifying amplicons are the primer pairs of Table 2. In other embodiments, other combinations of primer pairs are possible by combining certain members of the forward primers with certain members of the reverse primers. An example can be seen in Table 2 for two primer pair combinations of forward primer 165_EC_789_810_F (SEQ ID NO: 206), with the reverse primers 165_EC_880_894_R (SEQ ID NO: 796), or 165_EC_882_899_R or (SEQ ID NO: 818). Arriving at a favorable alternate combination of primers in a primer pair depends upon the properties of the primer pair, most notably the size of the bioagent identifying amplicon that would be produced by the primer pair, which preferably is between about 45 to about 150 nucleobases in length. Alternatively, a bioagent identifying amplicon longer than 150 nucleobases in length could be cleaved into smaller segments by cleavage reagents such as chemical reagents, or restriction enzymes, for example.

- [315] In some embodiments, the primers are configured to amplify nucleic acid of a bioagent to produce amplification products that can be measured by mass spectrometry and from whose molecular masses candidate base compositions can be readily calculated.
- [316] In some embodiments, any given primer comprises a modification comprising the addition of a non-templated T residue to the 5' end of the primer (i.e., the added T residue does not necessarily hybridize to the nucleic acid being amplified). The addition of a non-templated T residue has an effect of minimizing the addition of non-templated adenosine residues as a result of the non-specific enzyme activity of Taq polymerase (Magnuson et al., Biotechniques, 1996, 21, 700-709), an occurrence which may lead to ambiguous results arising from molecular mass analysis.
- [317] In some embodiments of the present invention, primers may contain one or more universal bases. Because any variation (due to codon wobble in the 3rd position) in the conserved regions among species is likely to occur in the third position of a DNA (or RNA) triplet, oligonucleotide primers can be designed such that the nucleotide corresponding to this position is a base which can bind to more than one nucleotide, referred to herein as a "universal nucleobase." For example, under this "wobble" pairing, inosine (I) binds to U, C or A; guanine (G) binds to U or C, and uridine (U) binds to U or C. Other examples of universal nucleobases include nitroindoles such as 5-nitroindole or 3-nitropytrole (Loakes et al., Nucleosides and Nucleotides, 1995, 14, 1003-1003), the degenerate nucleotides dP or dK (Hill et al.), an acyclic nucleoside analog containing 5-nitroindazole (Van Aerschot et al., Nucleosides and Nucleotides, 1995, 14, 1033-1056) or the purine analog 1-(2-deoxy-β-D-ribofuranosyl)-imidazole-4-carboxamide (Sala et al., Nucl. Acids Res., 1996, 24, 3302-3306).

[318] In some embodiments, to compensate for the somewhat weaker binding by the wobble base, the oligonucleotide primers are designed such that the first and second positions of each triplet are occupied by nucleotide analogs that bind with greater affinity than the unmodified nucleotide. Examples of these analogs include, but are not limited to, 2,6-diaminopurine which binds to thymine, 5-propynyluracil (also known as propynylated thymine) which binds to adenine and 5-propynylcytosine and phenoxazines, including G-clamp, which binds to G. Propynylated pyrimidines are described in U.S. Patent Nos. 5,645,985, 5,830,653 and 5,484,908, each of which is commonly owned and incorporated herein by reference in its entirety. Propynylated primers are described in U.S. Pre-Grant Publication No. 2003-0170682, which is also commonly owned and incorporated herein by reference in its entirety.

Phenoxazines are described in U.S. Patent Nos. 5,502,177, 5,763,588, and 6,005,096, each of which is incorporated herein by reference in its entirety. G-clamps are described in U.S. Patent Nos. 6,007,992 and 6,028,183, each of which is incorporated herein by reference in its entirety.

[319] In some embodiments, primer hybridization is enhanced using primers containing 5-propynyl deoxy-cytidine and deoxy-thymidine nucleotides. These modified primers offer increased affinity and base pairing selectivity.

[320] In some embodiments, non-template primer tags are used to increase the melting temperature (T_m) of a primer-template duplex in order to improve amplification efficiency. A non-template tag is at least three consecutive A or T nucleotide residues on a primer which are not complementary to the template. In any given non-template tag, A can be replaced by C or G and T can also be replaced by C or G. Although Watson-Crick hybridization is not expected to occur for a non-template tag relative to the template, the extra hydrogen bond in a G-C pair relative to an A-T pair confers increased stability of the primer-template duplex and improves amplification efficiency for subsequent cycles of amplification when the primers hybridize to strands synthesized in previous cycles.

[321] In other embodiments, propynylated tags may be used in a manner similar to that of the nontemplate tag, wherein two or more 5-propynylcytidine or 5-propynyluridine residues replace template matching residues on a primer. In other embodiments, a primer contains a modified internucleoside linkage such as a phosphorothioate linkage, for example.

[322] In some embodiments, the primers contain mass-modifying tags. Reducing the total number of possible base compositions of a nucleic acid of specific molecular weight provides a means of avoiding a persistent source of ambiguity in determination of base composition of amplification products. Addition of mass-modifying tags to certain nucleobases of a given primer will result in simplification of de novo determination of base composition of a given bioagent identifying amplicon from its molecular mass.

[323] In some embodiments of the present invention, the mass modified nucleobase comprises one or more of the following: for example, 7-deaza-2'-deoxyadenosine-5-triphosphate, 5-iodo-2'-deoxyuridine-5'-triphosphate, 5-bromo-2'-deoxyuridine-5'-triphosphate, 5-iodo-2'-deoxyuridine-5'-triphosphate, 5-iodo-2'-deoxyuridine-5'-triphosphate, 5-triphosphate, 5-triphosphate, 5-triphosphate, 5-triphosphate, 5-triphosphate, 5-triphosphate, 5-triphosphate, 6-fluoro-2'-deoxyuridine-5'-triphosphate, 0'-deoxyuridine-5'-triphosphate, N2-methyl-2'-deoxyuridine-5'-triphosphate, 8-oxo-2'-deoxyuridine-5'-triphosphate, N2-methyl-2'-deoxyuridine-5'-triphosphate, 8-oxo-2'-deoxyuridine-5'-triphosphate, N1-methyl-2'-deoxyuridine-5'-triphosphate, N2-methyl-2'-deoxyuridine-5'-triphosphate, N-oxo-2'-deoxyuridine-5'-triphosphate, N1-methyl-2'-deoxyuridine-5'-triphosphate, N-oxo-2'-deoxyuridine-5'-triphosphate, N1-methyl-2'-deoxyuridine-5'-triphosphate, N-oxo-2'-deoxyuridine-5'-triphosphate, N1-methyl-2'-deoxyuridine-5'-triphosphate, N-oxo-2'-deoxyuridine-5'-triphosphate, N-oxo-2'-deo

[324] In some embodiments, multiplex amplification is performed where multiple bioagent identifying amplicons are amplified with a plurality of primer pairs. The advantages of multiplexing are that fewer reaction containers (for example, wells of a 96- or 384-well plate) are needed for each molecular mass measurement, providing time, resource and cost savings because additional bioagent identification data can be obtained within a single analysis. Multiplex amplification methods are well known to those with ordinary skill and can be developed without undue experimentation. However, in some embodiments, one useful and non-obvious step in selecting a plurality candidate bioagent identifying amplicons for multiplex amplification is to ensure that each strand of each amplification product will be sufficiently different in molecular mass that mass pectral signals will not overlap and lead to ambiguous analysis results. In some embodiments, a 10 Da difference in mass of two strands of one or more amplification products is sufficient to avoid overlap of mass spectral peaks.

[325] In some embodiments, as an alternative to multiplex amplification, single amplification reactions can be pooled before analysis by mass spectrometry. In these embodiments, as for multiplex amplification embodiments, it is useful to select a plurality of candidate bioagent identifying amplicons to ensure that each strand of each amplification product will be sufficiently different in molecular mass that mass spectral signals will not overlap and lead to ambiguous analysis results.

C Determination of Molecular Mass of Bioagent Identifying Amplicons

[326] In some embodiments, the molecular mass of a given bioagent identifying amplicon is determined by mass spectrometry. Mass spectrometry has several advantages, not the least of which is high bandwidth characterized by the ability to separate (and isolate) many molecular peaks across a broad range of mass to charge ratio (m/z). Thus mass spectrometry is intrinsically a parallel detection scheme without the need for radioactive or fluorescent labels, since every amplification product is identified by its molecular mass. The current state of the art in mass spectrometry is such that less than femtomole quantities of material can be readily analyzed to afford information about the molecular contents of the

sample. An accurate assessment of the molecular mass of the material can be quickly obtained, irrespective of whether the molecular weight of the sample is several hundred, or in excess of one hundred thousand atomic mass units (amu) or Daltons.

[327] In some embodiments, intact molecular ions are generated from amplification products using one of a variety of ionization techniques to convert the sample to gas phase. These ionization methods include, but are not limited to, electrospray ionization (ES), matrix-assisted laser desorption ionization (MALDI) and fast atom bombardment (FAB). Upon ionization, several peaks are observed from one sample due to the formation of ions with different charges. Averaging the multiple readings of molecular mass obtained from a single mass spectrum affords an estimate of molecular mass of the bioagent identifying amplicon. Electrospray ionization mass spectrometry (ESI-MS) is particularly useful for very high molecular weight polymers such as proteins and nucleic acids having molecular weights greater than 10 kDa, since it yields a distribution of multiply-charged molecules of the sample without causing a significant amount of fragmentation.

[328] The mass detectors used in the methods of the present invention include, but are not limited to, Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), time of flight (TOF), ion trap, quadrupole, magnetic sector, O-TOF, and triple quadrupole.

D. Base Compositions of Bioagent Identifying Amplicons

[329] Although the molecular mass of amplification products obtained using intelligent primers provides a means for identification of bioagents, conversion of molecular mass data to a base composition signature is useful for certain analyses. As used herein, "base composition" is the exact number of each nucleobase (A, T, C and G) determined from the molecular mass of a bioagent identifying amplicon. In some embodiments, a base composition provides an index of a specific organism. Base compositions can be calculated from known sequences of known bioagent identifying amplicons and can be experimentally determined by measuring the molecular mass of a given bioagent identifying amplicon, followed by determination of all possible base compositions which are consistent with the measured molecular mass within acceptable experimental error. The following example illustrates determination of base composition from an experimentally obtained molecular mass of a 46-mer amplification product originating at position 1337 of the 165 rRNA of Bacillus anthracis. The forward and reverse strands of the amplification product have measured molecular masses of 14208 and 14079 Da, respectively. The possible base compositions derived from the molecular masses of the forward and reverse strands for the B. authracis products are listed in Table 1.

Possible Base Compositions for B. anthracis 46mer Amplification Product

		•		- k	
Calc. Mass	Mass Error	Base	Calc. Mass	Mass Error	Base
Forward	Forward	Composition of	Reverse	Reverse	Composition of
Strand	Strand	Forward Strand	Strand	Strand	Reverse Strand
14208.2935	0.079520	A1 G17 C10 T18	14079.2624	0.080600	A0 G14 C13 T19
14208.3160	0.056980	A1 G20 C15 T10	14079.2849	0.058060	A0 G17 C18 T11
14208.3386	0.034440	A1 G23 C20 T2	14079.3075	0.035520	A0 G20 C23 T3
14208.3074	0.065560	A6 G11 C3 T26	14079.2538	0.089180	A5 G5 C1 T35
14208.3300	0.043020	A6 G14 C8 T18	14079.2764	0.066640	A5 G8 C6 T27
14208.3525	0.020480	A6 G17 C13 T10	14079.2989	0.044100	A5 G11 C11 T19
14208.3751	0.002060	A6 G20 C18 T2	14079.3214	0.021560	A5 G14 C16 T11
14208.3439	0.029060	A11 G8 C1 T26	14079.3440	0.000980	A5 G17 C21 T3
14208.3665	0.006520	A11 G11 C6 T18	14079.3129	0.030140	A10 G5 C4 T27
14208.3890	0.016020	A11 G14 C11 T10	14079.3354	0.007600	A10 G8 C9 T19
14208.4116	0.038560	A11 G17 C16 T2	14079.3579	0.014940	A10 G11 C14 T11
14208.4030	0.029980	A16 G8 C4 T18	14079.3805	0.037480	A10 G14 C19 T3
14208.4255	0.052520	A16 G11 C9 T10	14079.3494	0.006360	A15 G2 C2 T27
14208.4481	0.075060	A16 G14 C14 T2	14079.3719	0.028900	A15 G5 C7 T19
14208.4395	0.066480	A21 G5 C2 T18	14079.3944	0.051440	A15 G8 C12 T11
14208.4620	0.089020	A21 G8 C7 T10	14079.4170 '	0.073980	A15 G11 C17 T3
-	-	-	14079.4084	0.065400	A20 G2 C5 T19
-	-	-	14079.4309	0.087940	A20 G5 C10 T13

[330] Among the 16 possible base compositions for the forward strand and the 18 possible base compositions for the reverse strand that were calculated, only one pair (shown in **bold**) are complementary base compositions, which indicates the true base composition of the amplification product. It should be recognized that this logic is applicable for determination of base compositions of any bioagent identifying amplicon, regardless of the class of bioagent from which the corresponding amplification product was obtained.

[331] In some embodiments, assignment of previously unobserved base compositions (also known as "true unknown base compositions") to a given phylogeny can be accomplished via the use of pattern classifier model algorithms. Base compositions, like sequences, vary slightly from strain to strain within species, for example. In some embodiments, the pattern classifier model is the mutational probability model. On other embodiments, the pattern classifier is the polytope model. The mutational probability model and polytope model are both commonly owned and described in U.S. Patent application Serial No. 11/073,362 which is incorporated herein by reference in entirety.

[332] In one embodiment, it is possible to manage this diversity by building "base composition probability clouds" around the composition constraints for each species. This permits identification of

organisms in a fashion similar to sequence analysis. A "pseudo four-dimensional plot" can be used to visualize the concept of base composition probability clouds. Optimal primer design requires optimal choice of bioagent identifying amplicons and maximizes the separation between the base composition signatures of individual bioagents. Areas where clouds overlap indicate regions that may result in a misclassification, a problem which is overcome by a triangulation identification process using bioagent identifying amplicons not affected by overlap of base composition probability clouds.

[333] In some embodiments, base composition probability clouds provide the means for screening potential primer pairs in order to avoid potential misclassifications of base compositions. In other embodiments, base composition probability clouds provide the means for predicting the identity of a bioagent whose assigned base composition was not previously observed and/or indexed in a bioagent identifying amplicon base composition database due to evolutionary transitions in its nucleic acid sequence. Thus, in contrast to probe-based techniques, mass spectrometry determination of base composition does not require prior knowledge of the composition or sequence in order to make the measurement.

[334] The present invention provides bioagent classifying information similar to DNA sequencing and phylogenetic analysis at a level sufficient to identify a given bioagent. Furthermore, the process of determination of a previously unknown base composition for a given bioagent (for example, in a case where sequence information is unavailable) has downstream utility by providing additional bioagent indexing information with which to populate base composition databases. The process of future bioagent identification is thus greatly improved as more BCS indexes become available in base composition databases.

E. Triangulation Identification

[335] In some cases, a molecular mass of a single bioagent identifying amplicon alone does not provide enough resolution to unambiguously identify a given bioagent. The employment of more than one bioagent identifying amplicon for identification of a bioagent is herein referred to as "triangulation identification." Triangulation identification is pursued by determining the molecular masses of a plurality of bioagent identifying amplicons selected within a plurality of housekeeping genes. This process is used to reduce false negative and false positive signals, and enable reconstruction of the origin of hybrid or otherwise engineered bioagents. For example, identification of the three part toxin genes typical of B. anthracis (Bowen et al., J. Appl. Microbiol., 1999, 87, 270-278) in the absence of the expected signatures from the B. anthracis genome would suggest a genetic engineering event.

[336] In some embodiments, the triangulation identification process can be pursued by characterization of bioagent identifying amplicons in a massively parallel fashion using the polymerase chain reaction (PCR), such as multiplex PCR where multiple primers are employed in the same amplification reaction mixture, or PCR in multi-well plate format wherein a different and unique pair of primers is used in multiple wells containing otherwise identical reaction mixtures. Such multiplex and multi-well PCR methods are well known to those with ordinary skill in the arts of rapid throughput amplification of nucleic acids. In other related embodiments, one PCR reaction per well or container may be carried out, followed by an amplicon pooling step wherein the amplification products of different wells are combined in a single well or container which is then subjected to molecular mass analysis. The combination of pooled amplicons can be chosen such that the expected ranges of molecular masses of individual amplicons are not overlapping and thus will not complicate identification of signals.

F. Codon Base Composition Analysis

[337] In some embodiments of the present invention, one or more nucleotide substitutions within a codon of a gene of an infectious organism confer drug resistance upon an organism which can be determined by codon base composition analysis. The organism can be a bacterium, virus, fungus or protozoan.

[338] In some embodiments, the amplification product containing the codon being analyzed is of a length of about 35 to about 200 nucleobases. The primers employed in obtaining the amplification product can hybridize to upstream and downstream sequences directly adjacent to the codon, or can hybridize to upstream and downstream sequences one or more sequence positions away from the codon. The primers may have between about 70% to 100% sequence complementarity with the sequence of the gene containing the codon being analyzed.

[339] In some embodiments, the codon base composition analysis is undertaken

[340] In some embodiments, the codon analysis is undertaken for the purpose of investigating genetic disease in an individual. In other embodiments, the codon analysis is undertaken for the purpose of investigating a drug resistance mutation or any other deleterious mutation in an infectious organism such as a bacterium, virus, fungus or protozoan. In some embodiments, the bioagent is a bacterium identified in a biological product.

[341] In some embodiments, the molecular mass of an amplification product containing the codon being analyzed is measured by mass spectrometry. The mass spectrometry can be either electrospray (ESI) mass spectrometry or matrix-assisted laser desorption ionization (MALDI) mass spectrometry.

Time-of-flight (TOF) is an example of one mode of mass spectrometry compatible with the analyses of the present invention.

[342] The methods of the present invention can also be employed to determine the relative abundance of drug resistant strains of the organism being analyzed. Relative abundances can be calculated from amplitudes of mass spectral signals with relation to internal calibrants. In some embodiments, known quantities of internal amplification calibrants can be included in the amplification reactions and abundances of analyte amplification product estimated in relation to the known quantities of the calibrants.

[343] In some embodiments, upon identification of one or more drug-resistant strains of an infectious organism infecting an individual, one or more alternative treatments can be devised to treat the individual.

G. Determination of the Quantity of a Bioagent

[344] In some embodiments, the identity and quantity of an unknown bioagent can be determined using the process illustrated in Figure 2. Primers (500) and a known quantity of a calibration polynucleotide (505) are added to a sample containing nucleic acid of an unknown bioagent. The total nucleic acid in the sample is then subjected to an amplification reaction (510) to obtain amplification products. The molecular masses of amplification products are determined (515) from which are obtained molecular mass and abundance data. The molecular mass of the bioagent identifying amplicon (520) provides the means for its identification (525) and the molecular mass of the calibration amplicon obtained from the calibration polynucleotide (530) provides the means for its identification (335). The abundance data of the bioagent identifying amplicon is recorded (540) and the abundance data for the calibration data is recorded (545), both of which are used in a calculation (550) which determines the quantity of unknown bioagent in the sample.

[345] A sample comprising an unknown bioagent is contacted with a pair of primers that provide the means for amplification of nucleic acid from the bioagent, and a known quantity of a polynucleotide that comprises a calibration sequence. The nucleic acids of the bioagent and of the calibration sequence are amplified and the rate of amplification is reasonably assumed to be similar for the nucleic acid of the bioagent and of the calibration sequence. The amplification reaction then produces two amplification products: a bioagent identifying amplicon and a calibration amplicon. The bioagent identifying amplicon and the calibration amplicon should be distinguishable by molecular mass while being amplified at essentially the same rate. Effecting differential molecular masses can be accomplished by choosing as a calibration sequence, a representative bioagent identifying amplicon (from a specific species of bioagent) and performing, for example, a 2-8 nucleobase deletion or insertion within the variable region between

the two priming sites. The amplified sample containing the bioagent identifying amplicon and the calibration amplicon is then subjected to molecular mass analysis by mass spectrometry, for example. The resulting molecular mass analysis of the nucleic acid of the bioagent and of the calibration sequence provides molecular mass data and abundance data for the nucleic acid of the bioagent and of the calibration sequence. The molecular mass data obtained for the nucleic acid of the bioagent enables calculation of the unknown bioagent and the abundance data enables calculation of the quantity of the bioagent, based on the knowledge of the quantity of calibration polynucleotide contacted with the sample.

- [346] In some embodiments, construction of a standard curve where the amount of calibration polynucleotide spiked into the sample is varied provides additional resolution and improved confidence for the determination of the quantity of bioagent in the sample. The use of standard curves for analytical determination of molecular quantities is well known to one with ordinary skill and can be performed without undue experimentation.
- [347] In some embodiments, multiplex amplification is performed where multiple bioagent identifying amplicons are amplified with multiple primer pairs which also amplify the corresponding standard calibration sequences. In this or other embodiments, the standard calibration sequences are optionally included within a single vector which functions as the calibration polynucleotide. Multiplex amplification methods are well known to those with ordinary skill and can be performed without undue experimentation.
- [348] In some embodiments, the calibrant polynucleotide is used as an internal positive control to confirm that amplification conditions and subsequent analysis steps are successful in producing a measurable amplicon. Even in the absence of copies of the genome of a bioagent, the calibration polynucleotide should give rise to a calibration amplicon. Failure to produce a measurable calibration amplicon indicates a failure of amplification or subsequent analysis step such as amplicon purification or molecular mass determination. Reaching a conclusion that such failures have occurred is in itself, a useful event.
- [349] In some embodiments, the calibration sequence is comprised of DNA. In some embodiments, the calibration sequence is comprised of RNA.
- [350] In some embodiments, the calibration sequence is inserted into a vector that itself functions as the calibration polynucleotide. In some embodiments, more than one calibration sequence is inserted into the vector that functions as the calibration polynucleotide. Such a calibration polynucleotide is herein termed a "combination calibration polynucleotide." The process of inserting polynucleotides into vectors

is routine to those skilled in the art and can be accomplished without undue experimentation. Thus, it should be recognized that the calibration method should not be limited to the embodiments described herein. The calibration method can be applied for determination of the quantity of any bioagent identifying amplicon when an appropriate standard calibrant polynucleotide sequence is designed and used. The process of choosing an appropriate vector for insertion of a calibrant is also a routine operation that can be accomplished by one with ordinary skill without undue experimentation.

H. Identification of Bacteria

[351] In other embodiments of the present invention, the primer pairs produce bioagent identifying amplicons within stable and highly conserved regions of bacteria. The advantage to characterization of an amplicon defined by priming regions that fall within a highly conserved region is that there is a low probability that the region will evolve past the point of primer recognition, in which case, the primer hybridization of the amplification step would fail. Such a primer set is thus useful as a broad range survey-type primer. In another embodiment of the present invention, the intelligent primers produce bioagent identifying amplicons including a region which evolves more quickly than the stable region described above. The advantage of characterization bioagent identifying amplicon corresponding to an evolving genomic region is that it is useful for distinguishing emerging strain variants or the presence of virulence genes, drug resistance genes, or codon mutations that induce drug resistance.

[352] The present invention also has significant advantages as a platform for identification of diseases caused by emerging bacterial strains such as, for example, drug-resistant strains of Staphylococcus aureus. The present invention eliminates the need for prior knowledge of bioagent sequence to generate hybridization probes. This is possible because the methods are not confounded by naturally occurring evolutionary variations occurring in the sequence acting as the template for production of the bioagent identifying amplicon. Measurement of molecular mass and determination of base composition is accomplished in an unbiased manner without sequence prejudice.

[353] Another embodiment of the present invention also provides a means of tracking the spread of a bacterium, such as a particular drug-resistant strain when a plurality of samples obtained from different locations are analyzed by the methods described above in an epidemiological setting. In one embodiment, a plurality of samples from a plurality of different locations is analyzed with primer pairs which produce bioagent identifying amplicons, a subset of which contains a specific drug-resistant bacterial strain. The corresponding locations of the members of the drug-resistant strain subset indicate the spread of the specific drug-resistant strain to the corresponding locations.

I. Kits

[354] The present invention also provides kits for carrying out the methods described herein. In some embodiments, the kit may comprise a sufficient quantity of one or more primer pairs to perform an amplification reaction on a target polynucleotide from a bioagent to form a bioagent identifying amplicon. In some embodiments, the kit may comprise from one to fifty primer pairs, from one to twenty primer pairs, from one to ten primer pairs, or from two to five primer pairs. In some embodiments, the kit may comprise one or more primer pairs recited in Table 2.

[355] In some embodiments, the kit comprises one or more broad range survey primer(s), division wide primer(s), or drill-down primer(s), or any combination thereof. If a given problem involves identification of a specific bioagent, the solution to the problem may require the selection of a particular combination of primers to provide the solution to the problem. A kit may be designed so as to comprise particular primer pairs for identification of a particular bioagent. A drill-down kit may be used, for example, to distinguish different genotypes or strains, drug-resistant, or otherwise. In some embodiments, the primer pair components of any of these kits may be additionally combined to comprise additional combinations of broad range survey primers and division-wide primers so as to be able to identify a bacterium.

[356] In some embodiments, the kit contains standardized calibration polynucleotides for use as internal amplification calibrants. Internal calibrants are described in commonly owned U.S. Patent Application Serial No: 60/545,425 which is incorporated herein by reference in its entirety.

[357] In some embodiments, the kit comprises a sufficient quantity of reverse transcriptase (if RNA is to be analyzed for example), a DNA polymerase, suitable nucleoside triphosphates (including alternative dNTPs such as inosine or modified dNTPs such as the 5-propynyl pyrimidines or any dNTP containing molecular mass-modifying tags such as those described above), a DNA ligase, and/or reaction buffer, or any combination thereof, for the amplification processes described above. A kit may further include instructions pertinent for the particular embodiment of the kit, such instructions describing the primer pairs and amplification conditions for operation of the method. A kit may also comprise amplification reaction containers such as microcentrifuge tubes and the like. A kit may also comprise reagents or other materials for isolating bioagent nucleic acid or bioagent identifying amplicons from amplification, including, for example, detergents, solvents, or ion exchange resins which may be linked to magnetic beads. A kit may also comprise a table of measured or calculated molecular masses and/or base compositions of bioagents using the primer pairs of the kit.

[358] Some embodiments are kits that contain one or more survey bacterial primer pairs represented by primer pair compositions wherein each member of each pair of primers has 70% to 100% sequence

identity with the corresponding member from the group of primer pairs represented by any of the primer pairs of Table 5. The survey primer pairs may include broad range primer pairs which hybridize to ribosomal RNA, and may also include division-wide primer pairs which hybridize to housekeeping genes such as rplB, tufB, rpoB, rpoC, valS, and infB, for example.

[359] In some embodiments, a kit may contain one or more survey bacterial primer pairs and one or more triangulation genotyping analysis primer pairs such as the primer pairs of Tables 8, 12, 14, 19, 21, 23, or 24. In some embodiments, the kit may represent a less expansive genotyping analysis but include triangulation genotyping analysis primer pairs for more than one genus or species of bacteria. For example, a kit for surveying nosocomial infections at a health care facility may include, for example, one or more broad range survey primer pairs, one or more division wide primer pairs, one or more Actnetobacter baumannii triangulation genotyping analysis primer pairs and one or more Staphylococcus aureus triangulation genotyping analysis primer pairs. One with ordinary skill will be capable of analyzing in silico amplification data to determine which primer pairs will be able to provide optimal identification resolution for the bacterial bioagents of interest.

[360] In some embodiments, a kit may be assembled for identification of strains of bacteria involved in contamination of food. An example of such a kit embodiment is a kit comprising one or more bacterial survey primer pairs of Table 5 with one or more triangulation genotyping analysis primer pairs of Table 12 which provide strain resolving capabilities for identification of specific strains of Campylobacter jejuni.

[361] Some embodiments of the kits are 96-well or 384-well plates with a plurality of wells containing any or all of the following components: dNTPs, buffer salts, Mg²⁺, betaine, and primer pairs. In some embodiments, a polymerase is also included in the plurality of wells of the 96-well or 384-well plates.

[362] Some embodiments of the kit contain instructions for PCR and mass spectrometry analysis of amplification products obtained using the primer pairs of the kits.

[363] Some embodiments of the kit include a barcode which uniquely identifies the kit and the components contained therein according to production lots and may also include any other information relative to the components such as concentrations, storage temperatures, etc. The barcode may also include analysis information to be read by optical barcode readers and sent to a computer controlling amplification, purification and mass spectrometric measurements. In some embodiments, the barcode provides access to a subset of base compositions in a base composition database which is in digital

communication with base composition analysis software such that a base composition measured with primer pairs from a given kit can be compared with known base compositions of bioagent identifying amplicons defined by the primer pairs of that kit.

[364] In some embodiments, the kit contains a database of base compositions of bioagent identifying amplicons defined by the primer pairs of the kit. The database is stored on a convenient computer readable medium such as a compact disk or USB drive, for example.

In some embodiments, the kit includes a computer program stored on a computer formatted medium (such as a compact disk or portable USB disk drive, for example) comprising instructions which direct a processor to analyze data obtained from the use of the primer pairs of the present invention. The instructions of the software transform data related to amplification products into a molecular mass or base composition which is a useful concrete and tangible result used in identification and/or classification of bioagents. In some embodiments, the kits of the present invention contain all of the reagents sufficient to carry out one or more of the methods described herein.

[366] While the present invention has been described with specificity in accordance with certain of its embodiments, the following examples serve only to illustrate the invention and are not intended to limit the same. In order that the invention disclosed herein may be more efficiently understood, examples are provided below. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the invention in any manner.

EXAMPLES

Example 1: Design and Validation of Primers that Define Bioagent Identifying Amplicons for Identification of Bacteria

[367] For design of primers that define bacterial bioagent identifying amplicons, a series of bacterial genome segment sequences were obtained, aligned and scanned for regions where pairs of PCR primers would amplify products of about 45 to about 150 nucleotides in length and distinguish subgroups and/or individual strains from each other by their molecular masses or base compositions. A typical process shown in Figure 1 is employed for this type of analysis.

[368] A database of expected base compositions for each primer region was generated using an in silico PCR search algorithm, such as (ePCR). An existing RNA structure search algorithm (Macke et al., Nucl. Acids Res., 2001, 29, 4724-4735, which is incorporated herein by reference in its entirety) has been modified to include PCR parameters such as hybridization conditions, mismatches, and thermodynamic calculations (SantaLucia, Proc. Natl. Acad. Sci. U.S.A., 1998, 95, 1460-1465, which is incorporated

herein by reference in its entirety). This also provides information on primer specificity of the selected primer pairs.

Table 2 represents a collection of primers (sorted by primer pair number) designed to identify [369] bacteria using the methods described herein. The primer pair number is an in-house database index number. Primer sites were identified on segments of genes, such as, for example, the 16S rRNA gene. The forward or reverse primer name shown in Table 2 indicates the gene region of the bacterial genome to which the primer hybridizes relative to a reference sequence. In Table 2, for example, the forward primer name 16S EC 1077 1106 F indicates that the forward primer (F) hybridizes to residues 1077-1106 of the reference sequence represented by a sequence extraction of coordinates 4033120..4034661 from GenBank gi number 16127994 (as indicated in Table 3). As an additional example: the forward primer name BONTA_X52066 450 473 indicates that the primer hybridizes to residues 450-437 of the gene encoding Clostridium botulinum neurotoxin type A (BoNT/A) represented by GenBank Accession No. X52066 (primer pair name codes appearing in Table 2 are defined in Table 3. One with ordinary skill knows how to obtain individual gene sequences or portions thereof from genomic sequences present in GenBank. In Table 2, Tp = 5propynyluracil; Cp = 5-propynylcytosine; * = phosphorothioate linkage; I = inosine. T. GenBank Accession Numbers for reference sequences of bacteria are shown in Table 3 (below). In some cases, the reference sequences are extractions from bacterial genomic sequences or complements thereof.

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Table 2: Primer Pairs for Identification of Bacteria

Reverse SEQ tence ID NO:	crc 809	TCCTC 1398	CCTC 1159	CCG 787	806	CCGC 897	CT 1365	1364	TCCC 839	CTA 798	830	830	796	735	734	308 808	1252	TTCBGC 846	ACCC 717	716	1282		PACGTAACG 1150	+	- g	o o	0
Reverse Sequence	GACGICATCCCCACCTICCTC	TTGACGTCATCCCCACCTTCCTC	TGACGTCATCCCCACCTTCCTC	CGAGTTGCAGACTGCGATCCG	GACGGGGGGTGTGTACAAG	TACGCATTACTCACCCGTCCGC	TTACTCACCCGTCCGCCGCT	TTACTCACCCGTCCGCC	GTATCTAATCCTGTTTGCTCCC	CGTGGACTACCAGGGTATCTA	GGCCGTACTCCCCAGGCG	GGCCGTACTCCCCAGGCG	CGTACTCCCCAGGCG	ACGAGCTGACGACAGCCATG	ACGACACGAGCTGACGAC	GACCGTTATAGTTACGGCC	TGCTTAGATGCTTTCAGC	GTTTCARGCTTAGATGCTTTCAGC	ACAAAAGGTACGCCGTCACCC	ACAAAGGCACGCCATCACCC	TGGCTGCTTCTAAGCCAAC		TGAATCTTGAAACACCATACGTAACG	TGAATCTTGAAACACCATACG	TGAATCTTGAAACACCAT TGAATCTTGAAACACAT GFAACCTTGTGAA	TGAATCTTGAACACOTACGTAACG TGAATCTTGAACACOATACG GFAACCCTTGTCTTTGAATTGAATTGA	TGAATCTTGAAACACCATT TGAACCCTTGTCTTGAA GGTAACCCTTGTCTTTGA TGGTAACCCTTGTCTTTGA
Reverse Primer Name	168 EC 1175 1195 R	168 EC 1175 1197 R	168 EC 1175 1196 R	16S EC 1303 1323 R	16S EC 1389 1407 R	168 BC 105 126 R	16S BC 101 120 R	16S EC 104 120 R	168 EC 774 795 R	16S BC 789 809 R	168 BC 880 897 R	16S EC 880 897 2 R	168 EC 880 894 R	168 BC 1054 1073 R	16S BC 1061 1078 R	238 BC 1906 1924 R	23S EC 2744 2761 R	238 BC 2751 2767 B	238 BC 551 571 R	23S EC 551 571 2 R	23S EC 1059 1077 R	CAPC BA 180 205 R		CAPC BA 185 205 R	CAPC BA 185 205 R	CAPC BA 185 205 R CAPC BA 349 376 R CAPC BA 358 377 R	CAPC BA 185 205 R CAPC BA 349 376 R CAPC BA 358 377 R CAPC BA 361 378 R
Forward SEQ ID NO:	134	38	651	114	10	429	136	152	137	21	118	119	206	672	13	90	408	83	125	125	99	139		20	109	109	20 109 663 138
Forward Sequence	GIGAGAIGINGGGFFAAGICCCGFAA	ATGTTGGGTTAAGTCCCGCAACGAG	TTBAGTCCCGCBACGATCGCBA	GCTACACACGCGTGCTACAATG	AAGTCGGAATCGCTAGTAATCG	TGAACGCTGGTGCCATGCTTAACAC	GTGGCATGCCTAATACATGCAAGTCG	TAACACATGCAAGTCGAACG	GIGINGCGGFGAAATGCG	AGAACACCGATGGCGAAGGC	GGATTAGAGACCCTGGTAGTCC	GGATTAGATACCCTGGTAGTCCACGC	TAGATACCCTGGTAGTCCACGC	TTCGATGCAACGCGAAGAACCT	ACGOGRAGACCTTACC	CTGACACCTGCCCGGTGC	TCTGTCCCTAGTACGAGAGGACCGG	DOOD ADD ADD ATO	GOGGAGTGAAAGAGATCCTGAAACCG	GGGGAGTGAAAGAGATCCTGAAACCG	CGAGAGGGAAACAACCCAGACC	GITATTIAGCACTCGTITTTAATCAG		ACTOSTITITA TORGCCCG	ACTOGETETETEATCAGCCCG GATTATTGTTATCCTGTTATGCCATT TGAG	ACTOSTITITANICAGECCO GATTATTGTTATCCFSTATGCCATT TGAG TTATTGTTATCCTGTTATGCC	ACTOSTITITATEMICAGE GATTATTOTTATCCNOTTATGGCCATT TGMG TTATTGTTATCCTGTTATGGCCATTATGGCATTATGGCATTATGGCATTATGGCATTATGGCCATTATGGCCATTATGGCCATTATGGCATTATGGCATTATGGCATTATGGCATTATGGCATTATGGCATTATGGCATTATGGCATTATGGCATTATGGCATTATGGCATTATGGCATTATGGCATTATGGCATTATGGCATTATGGCATTATGGCATT
Forward Primer Name	168 RC 1077 1106 F	163 BC 1082 1106 F	163 EC 1090 1111 F	168 EC 1222 1241 F	168 EC 1332 1353 F	168 EC 30 54 F	168 EC 38 64 F	168 EC 49 68 F	168 EC 683 700 F	168 EC 713 732 F	168 RC 785 806 F	16S EC 785 810 F	16S EC 789 810 F	16S EC 960 981 F	16S EC 969 985 F	23S EC 1826 1843 F	238 BC 2645 2669 F	238_BC_2645_2669_2_	238 RC 493 518 F	238 EC 493 518 2 F	238 EC 971 992 F	CAPC BA 104 131 F	E an outle	CAPC BA 114 100 F	CAPC BA 174 133 F	CAPC BA 274 303 F	CAPC BA 274 303 F CAPC BA 276 296 F CAPC BA 276 296 F
Primer Pair Number	T	6		4	ısı	9	7	8	6	1.0	1	12	13	1.4	1.5	16	17	;	0.1	20	21	22	00		52 25	24	25

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50	CYA BA 1349 1370 F	ACAACGAAGTACAATACAAGAC	12	CYA BA 1447 1426 R	CTTCTACATTTTTAGCCATCAC	800
		CGAAGTACAATACAAGACAAAAGAAG	,		COLO EL A COMMODO DE COMMODA	
30	CYA BA 1353 1379 F	9	54	CYA BA 1448 1467 K	TOTTOMORCELLCAMONCOC	77.77
31	CYA BA 1359 1379 F	ACAATACAAGACAAAAGAAGG	13	CYA BA 1447 1461 R	CGGCTTCAAGACCCC	794
32	CYA BA 914 937 F	CAGGITTAGTACCAGAACATGCAG	23	CYA BA 999 1026 R	ACCACTTTTAATAAGGTTTGTAGCTAAC	728
33	CYA BA 916 935 F	GGTTTAGTACCAGAACATGC	131	CYR. BR. 1003 1025 R	CCACTITIAATAAGGITTGTAGC	168
	e coct ayet De cant	TGCTCGTGGTGCACAAGTAACGGATA	F24	TNPR RC 1439 1467 R	TGCIGCITICGCAIGGTIAATIGCTICA	1248
35		TOBBOTAGRAPAGECO	254	LEF BA 1119 1135 R	GAATATCAATTTGTAGC	803
3,6	7 9901 9601 KH HR.	CARGARGARARAGECTTCTRARABG	44	LEF BA 1119 1149 R	AGATAAAGAATCACGAATATCAATTTGT AGC	745
37		AGCITTIGCATATTATATCGAGCCAC	26	LEF BA 843 872 R	TCTTCCAAGGATAGATTTATTTCTTGTT	1135
38	LEF BA 758 778 F	CTTTTGCATATTATATCGAGC	90	LEF BA 843 865 R	AGGATAGATTTATTTCTTGTTCG	748
39	LEF BA 795 813 F	TTTACAGCTTTATGCACCG	100	LEF BA 883 900 R	TCTTGACAGCATCCGTTG	1140
0.4	LEF BA 883 899 F	CAACGGATGCTGGCAAG	43	LEF BA 939 958 R	CAGATAAAGAATCGCTCCAG	762
41	PAG BA 122 142 F	CAGAATCAAGTTCCCAGGGG	49	PAG BA 190 209 R	CCTGTAGAAGAGGTAAC	781
42	PAG BA 123 145 F	AGAATCAAGTTCCCAGGGGTTAC	22	PAG BA 187 210 R	CCCTGTAGTAGAAGGTAACCAC	774
43	PAG BA 269 287 F	AATCTGCTATTTGGTCAGG	11	PAG BA 326 344 R	TGATTATCAGCGGRAGTAG	1186
44	PAG BA 655 675 F	GAAGGATATACGGTTGATGTC	93	PAG BA 755 772 R	COGTGCTCCATTTTTCAG	778
45	PAG BA 753 772 F	TCCTGAAAATGGAGCACGG	341	PAG BA 849 868 R	TOGGATAAGCTGCCACAAGG	1089
46	PAG BA 763 781 F	TGGAGCACGGCTTCTGATC	552	PAG BA 849 868 R	TCGGATAAGCTGCCACAAGG	1089
4.7	REDC RC 1018 1045 F		39	RPOC EC 1095 1124 R	TCAAGCGCCATTTCTTTTGGTAAACCAC AT	959
84	RPOC_EC_1018_1045_2	CAAAACTTATTAGGTAAGCGTGTTGA CT	39	RPOC EC 1095 1124 2 R	TCAAGCGCCATCTCTTCGGTAATCCAC	928
64	RPOC EC 114 140 F	TAAGAAGCCGGAAACCATCAACTACC	158	RPOC BC 213 232 R	GGCGCTTGTACTTACCGCAC	831
20	RPOC BC 2178 2196 F	-	478	RPOC EC 2225 2246 R	TIGGCCATCAGGCCACGCAIAC	1414
51	RPOC_BC_2178_2196_2	-	477	RPOC BC 2225 2246 2 R	TIGGCCATCAGACCACGCATAC	1413
122	RPOC EC 2218 2241 F	CTGGCAGGTATGCGTGGTCTGATG	8.1	RPOC EC 2313 2337 R	CGCACCGTGGGTTGAGATGAAGTAC	790
53	RFOC_BC_2218_2241_2	_	98	RPOC BC 2313 2337 2 R	CGCACCATGCGTAGAGATGAAGTAC	789
54	RPOC EC 808 833 F	COTCGGGTGATTAACCGTAACAACCG	75	RPOC EC 865 889 R	GTTTTTCGTTGCGTACGATGATGTC	847
55	RPOC EC 808 833 2 F	CGTCGTGTAATTAACCGTAACAACCG	2/2	RPOC BC 865 891 R	ACGTITITICGTITITICAACGATAATGCT	741
26	RPOC EC 993 1019 F	CARAGGTRAGCRAGGTCGTTTCCGTC A	41	RPOC EC 1036_1059_R	CONACCOCCTGAGTAGTCAACACG	785
	RPOC_EC_993_1019_2_	CAAAGGTAAGCAAGGACGTTTCCGTC	40	RPOC BC 1036 1059 2 R	CGAACGGCCAGAGTGAACACG	784

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SSPE BA 197 222 R	TUFB EC 283 303 R	TUFB EC 283 303 2 R	TUPB BC 1045 1068 R	TUFB BC 1045 1068 2 R	THTER RC 1033 1062 D	DDI:B DC 739 763 D	RPLB BC 736 757 R	RPOC EC 1097 1126 R	RPOB EC 3836 3865 R	RPLB EC 743 771 R	VALS EC 1195 1218 R	RPOB EC 1909 1929 R	RPLB EC 735 761 R	RPLB BC 737 762 R	a 311 00 117308 10108	a occ cre remain total	a ccc ooc remand total	Spin1 Spin1 255 380 0	SP101 SPET11 423 441 D	SP101 SPET11 448 473 R	SPI01 SPET11 686 714 P	SP101 SPET11 756 784 P	d 968 LLB LLLBds 101ds	SP101 SPET11 988 1012 R
45	204	678	4	10	4	86	54	78	248	54	7.7	233	623	169	2	118	7.0	80	132	126	62	127	364	123
CAAGCAAACGCACAATCAGAAGC	TAGACTGCCCAGGACACGCTG	TTGACTGCCCAGGTCACGCTG	AACTACCGTCCGCAGTTCTACTTCC	AACTACCGTCCTCAGTTCTACTTCC	CCACAGTTCTACTTCCGTACTACTGA	GACCTACAGTAAGAGGTTCTGTAATG	CATCCACACGGTGGTGGTGAAGG	CGTGTTGACTATTCGGGGGGGTTCAG	TCPACAACCICITGGAGGIPAAGCIC	CATCCACACGGTGGTGGTGAAGG	CGIGGCGCGTGGTTATCGA	TATCGCTCAGGCGAACTCCAAC	TGTAATGAACCCTAATGACCATCCAC	TAATGAACCCTAATGACCATCCACAC	AACCTTAATTGGAAAGAAACCCAAGA	GCTGGTGAAATAACCCAGATGTCGT	AGCAGGTGGTGAAATCGGCCACATGA	CITGIACTIGIGCICACACGGCTGT	GTChaagraccargrancraac	GGGGATTCAGCCATCAAAGCAGCTAT	CCTTACTTCGAACTATGAATCTTTTG	GGGGATTGATATCACCGATAAGAGA	TOGCCAATCAAAACTAAGGGAATGGC	GGGCAACAGCAGCGGATTGCGATTGC
SSPE BA 115 137 F	TUFE EC 239 259 F	TUFB EC 239 259 2 F	TUFB EC 976 1000 F	TUFB_EC_976_1000_2_ F	TURB BC 985 1012 F	RPLB EC 650 679 F	RPLB EC 688 710 F	RPOC EC 1036 1060 P	RPOB EC 3762 3790 F	RPLB EC 688 710 F	VALS EC 1105 1124 F	RPOB EC 1845 1866 F	RPLH EC 669 698 F	RPLB EC 671 700 P	SPIOI SPETII 1 29 F	SP101_SPET11_118_14	SP101_SPET11_216_24	SP101_SPET11_266_29 5 F	SP101_SPET11_322_34	SP101 SPET11 358 38	SP101_SPET11_600_62	SP101_SPET11_658_68	SP101_SPET11_776_80	SP101_SPET11_893_92
58	65	09	19	62	63	99	67	89	69	70	7.7	72	73	74	75	92	-11	78	79	80	81	83	83	4.6

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804	711	828	752	821	1412	755	1131	747	770	827	715	769	832	797	822	1386	930	792	1075	819	1389	733	727	727	740	
GACCCCAACCTGGCCTTTGTCGTTGA	AAACTAITITTTTAGCTATACTCGAACA C	GGATAATTGGTCGTAACAAGGGATAGTG AG	ATATGATTATCATTGAACTGCGGCCG	GOGTGACGACCTTCAATTGTAATCA	TTGGACCTGTAATCAGCTGAATACTGG	ATTGCCCAGAAATCAAATCATC	TCTGGGTGACCTGGTGTTTTAGA	AGCTGCTAGATGAGCTTCTGCCATGGCC	CCATAAGGTCACCGTCACCATTCAAAGC	GGAATTTACCAGCGATAGACACC	AATCGACGACCATCTTGGAAAGATTTCT C	CCAGCAGTTACTGTCCCCTCATCTTTG	GOGTCTACACCTGCACTTGCATTAAC	CGIATAAGCIGCACCAIAAGCIIGIAAT GC	GCGITCCACAGCTIGITGCAGAAG	Trescretesections	TATAGCACCATCTGAGGGGCAC	CGCGGTCGGTTGATGA	TOGCAGTTCATCAGCAGGAGGG	GOGCTCCACGTCTTCACGC	TTOGIGCTTAGATGCTTTCAG	ACGACACGAGCPTPGACGAC	ACACCAGCOTOGAC	ACACGAGCTGAC	AGGICCTICATCGCCTCIGA	
SP101 SPET11 1251 1277 R	SP101 SPET11 1403 1431 R	SP101 SPET11 1486 1515 R	SP101 SPET11 1783 1808 R	SP101 SPET11 1808 1835 R	SP101 SPET11 1901 1927 R	SP101 SPET11 2062 2083 R	SP101 SPET11 2375 2397 R	SP101 SPET11 2470 2497 R	SP101 SPET11 2543 2570 R	SP101 SPET11 3023 3045 R	SP101 SPET11 3168 3196 R	SP101 SPET11 3480 3506 R	SP101 SPET11 3605 3629 R	BEOR BC 3829 3848 B	VALS EC 1920 1943 R	RPOB EC 1438 1455 R	TUFB EC 284 309 R	DNAK BC 503 522 R	VALS EC 1948 1970 R	TUFB EC 849 867 R	23S RC 2745 2765 R	168 EC 1061 1078 2P R	16S EC 1064 1075 2P R	16S EC 1064 1075 R	23S BC 40 59 R	99
- 43	89	67	09	82	33	155	20	390	35	15	108	25	116	87	65	97	111	72	85	9	84	19	63	63	19	9
Characeanacacacacacacacacacacacacacacacacaca	CGCABABAATCCAGCTATTAGC	CGACTATAGCTAAAAAAATAGTITAT	CCTATATTAATCGTTTACAGAAACTG	CIGGCIAAACTITGGCAACGGI	ATGATTACAATTCAAGAAGGTOGTCA CGC	TAACGGTTATCATGGCCCAGATGGG	CAGAGACCGTTTTATCCTATCAGC	TCTAAAACACCAGGTCACCCAGAAG	ATGGCCATGGCAGAAGCTCA	ACCATGACAGAAGGCATTTTGACA	GATGACITITIAGCIAATGGTCAGGC	AGOGTAAAGGTGAACCTT	Dennes and a transfer of the second s	CTTGGAGGTAAGTCTCATTTTGGTGG	CGACGCTGCGCTTCAC	GACCACCTCGGCAACCGT	GCACTATGCACACGTAGATTGTCCTG	CGGCGTACTTCAACGACAGCCA	CTTCTGCAACAAGCTGTGGAACGC	ANGACGACCTGCACGGGC	CTGTTCTTAGTACGAGGGCC	ACGCGAAGAACCTTACDC	CGRAGRACDCDTTACC	CGRAGRACCTTACC	CCTGATAAGGGTGAGGTCG	
SP101 SPET11 1154 1	SP101 SPET11 1314 1	SPET11_1408_1	SPET11_1688_1	SP101_SPET11_1711_1	SPET11_1807_1	SP101_SPET11_1967_1 991 F	SP101_SPET11_2260_2 283 F	SP101 SPET11 2375 2 399 F	SP101_SPET11_2468_2 487 F	SP101_SPET11_2961_2	SP101_SPET11_3075_3	SP101_SPET11_3386_3	SP101 SPRT11 3511 3	0.000 arre 2000	VAT-S RC 1833 1850 F		TUFB EC 225 251 F	DNAK EC 428 449 F	VALS EC 1920 1943 F	TUPB EC 757 774 F	235 BC 2646 2667 F	16S BC 969 985 1P F	BC 972	972 985	TRNA_ILE- RRNH_EC 32 50.2 F	
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123	124	125	126	137	128	120	131	132	133	134	135	137	138	139	140	141	142	143	147	158	159	215	320	221	222	223	22.4	225	226	227	228	229	230	231

808	833	1385	782	094	775	346	/40	777	1369	841	742	801	839	835	757	71.4	21.4	0001	2004	192	783	731	773	817	818	810	749	750	751	838	750	751	030	000	750	751	838	838	
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ATAMGCCGGGTTCTGTCG	ATMAGCCATGTTCTGTTCCATC	AAGGAGGTGATCCAGCC	GTATCTAATCCTGTTTGCTCCC	TGACGTCATGCCCACCTTCC	TGACGTCATGGCCACCTTCC	AGACCTCCTGCGTGCAAAGC	TGACGTCATCCCCACCTTCC	AGTCCATCCCGGTCCTCTCG	GACGGGGGGTGTGTACAAG	CGAGTTGCAGACTGCGATCCG	COTACTOCCCAGGOG	ACGACACGAGCTGACGAC	CTICIACATITITAGCCATCAC	TGACGTCATCCCCACCTTCCTC	CGGCTGCTGGCACGAAGTTAG	TAGCCGCGGTCGAATTGCAT	CCGCGGTCGAATTGCATGCCTTC	CGACTIGACGGITAACATITCCTG	GTCCGACTTGACGGTCAACATTTCCTG	ACGCCATCAGGCCACGCAT	ACGGCACGAGGTAGTCGC	GAGCRICAGOGIGOGIGCT	GGCATCACCATTTCCTTGTCCTTCG	TGTTACTCACCGGTCTGCCACT	TATAACGCACATCGTCAGGGTGA	ACAACCATGCACCACCTGTC	TOGTGGACTACCAGGGTATCTA	TGGCCGTACTCCCCAGGCG	TACGAGCTGACGACCATG
RNBSEP EC 345 362 R	RNASEP SA 358 379 R	16S EC 1525 1541 R	16S EC 774 795 R	16S EC 1177 1196 10G R	16S_EC_1177_1196_10G_11G_R	TRNA ALA- RRNH EC 30 49 F MOD	16S EC 1177 1196 R MOD	23S BC 2658 2677 R MOD	168 BC 1389 1407 R	16S EC 1303 1323 R	16S EC 880 894 R	16S EC 1061 1078 R	CYA BA 1426 1447 R	16S EC 1175 1196 R	16S EC 507 527 R	GROL EC 577 596 R	GROL RC 571 593 R	RPOB EC 3862 3885 R	RPOB EC 3862 3888 R	RPOC EC 2227 2245 R	ASPS EC 521 538 R	RPOC EC 1437 1455 R	TUFB EC 1034 1058 R	16S BC 101 122 R	VALS EC 705 727 R	16S EC 1043 1062 R	16S EC 789 809 TWOD R	16S EC 880 897 TWOD R	16S EC 1054 1073 TMOD R
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RAMASED EC 61 77 F	T	1082 1100 F	+	9 0011 000 00	+	-	-		╌	EC 683 700 F	20 49 68 70	EC 49 68 F	RA 1349 1370 F	EC_1090_1111_2_	B 600 000 000	CDOT. DO 406 618 P	CDOF. WC C11 526 F	B 1000 0000 DB BOMB	RIVUE EC 3802 3821 F	B 1216 2116 DE DOUG	ASPS RC 405 422 F	D.		169 EC 7 33 F	VATS RC 610 649 F	168 EC 971 990 F	168_EC_713_732_TWDD	168_EC_785_806_TMOD	\vdash
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TGACCGTTATAGTTACGGCC	TGTAACCCTTGTCTTTGAATTGTATTTG	D	TTGTTAACGGCTTCAAGACCC	TIGCIGCITICGCAIGGITAATIGCITC	AA	TTCTTCCAAGGATAGATTTATTTCTTGT TCG	これ工程を成むしてあるのであるのであっているので	100000000000000000000000000000000000000	TTGCACGTCTGTTTCAGTTGCAAATTC	TTCCAAGTGCTGGTTTACCCCATGG	TGTGCTGGTTTACCCCATGGAGT	TCGGTACGAACTGGATGTCGCCGTT	TOCTGGATTCGCCTTTGCTACG	TITICGLIGCTTAGALGCTLTCAG	TIGACGICATCCCCACCITCCTC	TOTCCGACTTGACGGTCAACATTTCCTG	TACGCCATCAGGCCACGCAT		TGAGCATCAGCGTGCGTGCT	TOGGATCACCATTTCCTTGTCCTTCG	TCATGACAGCCAAGACCTCACCCACC	TGACCCCAACCTGGCCTTTTGTCGTTGA	TYGICGCCGATITCACCACCIGCTCCT	TAAACTATTITITIAGCTATACTCGAAC	TGGATAATTGGTCGTAACAAGGGATAGT	
23S BC 1906 1924 TMOD R	T	CAPC BA 349 376 TMOD R	CYA BA 1448 1467 TWOD R	t	INFB BC 1439 1467 TWOD R	LRF BA 843 872 TMOD R	6	KPOC 8C 4313 4321 4000 K	SSPE BA 197 222 TWOD R	RPLB EC 739 762 IMOD R	RPLB BC 736 757 TMOD R	VALS EC 1195 1218 TWOD R	RPOB EC 1909 1929 TWOD R	23S EC 2745 2765 TMOD R	168 EC 1175 1196 TMOD R	RPOB EC 3862 3888 TWOD R	BENCH EC 2222 2245 TWOD R	AFOC DC SES SES SES	RPOC EC 1437 1455 TWOD R	TUPB EC 1034 1058 TMOD R	SPIO1_SPET11_988_1012_IMO	SP101_SPET11_1251_1277_TM OD_R	SP101_SPET11_213_238_TMOD	SP101 SPET11 1403 1431 TM	SPIO1 SPET11 1486 1515 TM	00 k
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23S EC 1826 1843 TM	+	CAPC_BA_274_303_TMO	BA 1353 1379 TM		INFB_EC_1365_1393_T	A_756_781_TMOD	· .	MOD F	SSPE_BA_115_137_TWO D F	RPIB_EC_650_679_TWO	RPIB_EC_688_710_TMO	VALS EC 1105 1124 T	RPOB EC 1845 1866 T	238_BC_2646_2667_TM	168 RC 1090 1111 2	RPOB_EC_3799_3821_T	RPOC_EC_2146_2174_T	MOD F	NOD F	TURB BC 957 979 TWO	SP101 SPET11 893 92	SP101 SPET11 1154 1	SP101 SPET11 118 14	7 TMOD F SP101 SPET11 1314 1	SPIOI SPETLI 1408 1	437 TMOD F
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TATATGATTATCATTGAACTGCGGCCG	TGCGTGACGACCTTCTTGAATTGTAATC A	TITIGGACCIGIAAICAGCIGAATACIGG	TATTGCCCAGRANTCRATCRTC	TIGCCACITIGACAACTCCIGITGCIG	TTCTGGGTGACCTGGTGTTTTAGA	TAGCTGCTAGATGAGCTTCTGCCATGGC	TCCATAAGGTCACCGTCACCATTCAAAG C	TGCTGCTTTGATGGCTGAATCCCCTTC	TGGAATTTACCAGCGATAGACACC	TANTOGACGACCATCTTGGAAAGATTTC TC	TATCCCCTGCTTCTGCTGCC	TCCAGCAGTTACTGTCCCCTCATCTTTG	TGGGTCTACACCTGCACTTGCATAAC	TCCAACCTTTTCCACAGAATCAGC	TCCCATITITICAGGCATGCTGAAATA TC	TGATTGGCGATAAAGTGATATTTTCTAA AA	TCCCCACCAGAAAGACTAGCAGGATAA	TCCTACCCAACGTTCACCAAGGGCAG	TACCTITTCCACAACAGAATCAGC	TCGACGACCATCTTGGRAAGATTTC	TGTGCTGGTTTACCCCATGGAG	TGTTACTGCTGGAT	
SP101_SPET11_1783_1808_TM OD_R	SPIOL SPRILL 1808 1835 TM	SPI01_SPET11_1901_1927_TM	SPIOI SPETII 2062 2083 TM	SPIOI SPETIL 308 333 TWOD	SP101_SPET11_2375_2397_TM CD_R	SP101 SPET11 2470 2497 TM OD R	SPIOL SPETIL 2543 2570 TM	SP101_SPET11_355_380_TMOD R	SP101_SPET11_3023_3045_TM OD R	SP101_SPET11_3168_3196_TM OD R	SPIOL_SPETIL_423_441_TMOD	SP101_SPET11_3480_3506_TM OD R	SP101_SPET11_3605_3629_TM	SPIOL_SPETIL_448_473_TWOD	SP101_SPET11_686_714_TWOD	SP101_SPET11_756_784_TMOD R	SP101_SPET11_871_896_TMOD R	SPIO1_SPET11_92_116_TMOD_	SP101 SPET11 448 471 R	SP101 SPET11 3170 3194 R	RPLB EC 737 758 R	BONTA X52066 647 660 R	7.1
334	406	235	649	210	272	675	238	417	183	473	631	215	531	588	348	583	673	154	276	216	309	239	
SPI01_SPET11_1688_1 TCCTATATTAATCGTTTACAGAAACT 716_TMOD_F	TCTGGCTAAACTTTGGCAACGGT	TATGATTACAATTCAAGAAGGTOGTC	TTAACGGTTATCATGGCCCAGATGGG	TAGCAGGTGGAAATCGGCCACATG	TCAGAGACCGTTTTATCCTATCAGC	TTCTRABACACCAGGTCACCCAGAAG	TATGGCCATGGCAGAAGCTCA	TCTTGTACTTGTGGCTCACACGGCTG	TACCATGACAGAAGGCATTTTGACA	TGATGACTTTTTAGCTAATGGTCAGG	TGTCAAAGTGGCACGTTTACTGGC	TAGCGTAAAGGTGAACCTT	TGCTTCAGGAATCAATGATGGAGCAG	TGGGGATTCAGCCATCAAAGCAGCTA	TCCTTACTTCGAACTATGAATCTTTT	TOGGGATTGATATCACCGATAAGAAG	TTCGCCAATCAAAACTAAGGGAAATGG C	TANCCTIANTIGGAAAGAAACCCAAG	TCAGCCATCAAAGCAGCTATTG	TAGCTAATGGTCAGGCAGCC	TCCACACGGTGGTGGTGAAGG	TATEGCTCTACTCAA	
SP101_SPET11_1688_1 716_TMOD_F	SP101_SPET11_1711_1	SP101_SPET11_1807_1	SP101_SPET11_1967_1	3 TMOD F	SP101 SPET11 2260 2 283 TWOD F	SP101_SPET11_2375_2	SP101_SPET11_2468_2 487_TMOD_F	SP101 SPET11 266 29	SP101_SPET11_2961_2 984_TMOD_F	SP101 SPET11 3075 3	SP101_SPET11_322_34	SP101_SPET11_3386_3	SP101 SPET11 3511 3	SP101 SPET11 358 38	SP101_SPET11_600_62	SP101_SPET11_658_68	SP101_SPET11_776_80	SPIOI SPETII 1 29 T MOD F	SP101_SPET11_364_38	SP101 SPET11 3085 3	RPLB EC 690 710 F	BONTA_X52066_538_55	
428	429	430	431	432	433	7.7	435	T	Т	438	439	640	441	442	443	444	445	446	447	448	449	481	

1146	1367	1359	859	857	992	1241	1338	TAT	995	1401	1431	1202	1338	1190	366	1421	986	1402		1313	1304	943	879	1235	1053	1183
TG*Tp*TpA*Cp*TpG*Cp*TpGGAT	TIACITICIAACCCACTC	TTR*Cp*Tp*Tp*Cp*TpAA*Cp*Cp*C pA*Cp*TpC	TAACCATTTCGCGTAAGATTCAA	TAACCA*Tp*Tp*Tp*CpGCGTAAGA*T p*Tp*CpAA	TCATGTGCTAATGTTACTGCTGGATCTG	TGCpAGCpTGATpTpGT	TGTGCTpTpGMATpGCpT	TGATTGITTTGCpAGCpTGATpTpGT	TCATTTGTGTPTPTPGAATPGCPT	Tiechceicpiperincesine	TTCACAGCALGCACGLCIGITCAGII	TGCAGCTGATTGT	TGTGCTTTGAATGCT	TGATTGTTTTGCAGCTGATTGT	TCATTTGTGCTTTTGAATGCT	TIGHGATIGITITIGCAGCTGATIGTG	TCATAACTAGCATTTGTGCTTTGAATGC T	TTGCACGTCTGTTTCAGTTGCAAATTC	TOTABATTCCGCAAAGACTTTGGCATTA	9	TGGICTGAGIACCICCITIGC	TATTGGAAATACCGGCAGCATCTC	TAATGCGATACTGGCGTGCAAGTC	TGCGGGCTGGTTCAACAAGAG	TCCTGTTTTATAGCCGCCAAGAGTAAG	TOATGCGGGCTGGTTCAAC
BONTA X52066 647 660P R	BONTA X52066 759 775 R	BONTA X52066 759 775P R	BONTA X52066 517 539 R	BONTA X52066 517 539P R	BONTA X52066 644 671 R	SSPE BA 243 255P R	SSPE BA 163 177P R	SSPE BA 243 264P R	SSPE BA 163 182P R	SSPE BA 196 222P R	SSPR BA 202 231 R	GGDR RA 243 255 R	SSPE BA 163 177 R	SSPE BA 243 264 R	GCDP BA 163 182 B	SCOR BD 242 267 R	8 161 191 B	200 200 By 100 000 B	SSFE BR 130 SEE B	PLA AP053945 7434 7462 R	PLA AF053945 7482 7502 R	PLA AP053945 7539 7562 R	PLA AF053945 7257 7280 R	CAFL AF053947_33494_33514	CAPL_AP053947_33595_33621_R	CAP1_AF053947_33499_33517 R
143	94	91	393	142	463	616	192	533	602	255	488	613	179	533	009	900		010	255	442	327	481	657	292	270	542
DONTA_X52066_538_55 TA*TPGGC*TP*CP*TPA*CP*TP*C	Castragrastrastropage	GAA*TPAG*CPAA*TP*TP*C	Open para ama ama ama ama ama ama ama ama ama	T*Cp*TpAGTAATAATAGGA*Cp*Cp	TONGTCACTTGAAGTTGATACAAATC	TEGTOGCOTOAGCOATT	TACDAGAGTPTPTPGCPGAC	TRACTICINGGIDGCDIDAGCDAIT	TGGTACPAGAGTPTPTPGCpGAC	TOBRECORDANDEDGERGE	TGCACAATCAGAAGCTAAGAAAGCGC	ANGCI	Tegreciaecari	TACAGAGITITGCGAC	TOCTTOTOGTOCTOCTT	TGGTACAGAGTTTGCGAC	TGCAAGCTTCTGGTACAGAGTTTGCGA	U	TCAAGCAAACGCACAATCAGAAGC	TGACATCCGGCTCACGTTATTATGGT	TCCGGCTCACGTTATTATGGTAC	TGCAAAGGAGGTACTCAGACCAT	SAN	TATION OF THE PROPERTY OF THE PARTY OF THE P	TCACTCTTACATATAAGGAAGGCGCT	TGGRACTATTGCRACTGCTAATG
BONTA_X52066_538_55	X52066_701_72	A_XS2066_701_72	X52066_450_47	3 F BONTA_X52066_450_47	x52066_591_62	RD 146 1689 F	╁	150 1680 F	+	000m bh 114 127B F		SSPE BA 123 153 F	SSPE BA 156 168 F	SSPE BA 75 89 F	SSPE BA ING THE F	SSPE BA 72 89 F	SSPE BA 146 168 P	SSPE BA 63 89 F	SSPE BA 114 137 F	PLA_AP053945_7377_7	PLA AP053945_7382_7	PLA AP053945 7481 7	PLA_AF053945_7186_7	CAF1_AF053947_33407	CAF1 AF053947 33515	33457 F
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TCAAGGTTCTCACCGTTTACCTTAGGAG	TGITAAGTGTGTGCGGCTGTCTTATT	TCACGCGACGAGTGCCATCCATTG	TGACCCAAAGCTGAAAGCTTTACTG	THREGINGCAGATTATCTTTACCAA	TCTCATCCCGATATTACCGCCATGA	TGGCAACAGCTCAACACCTTTGG	TTCCAAGTGCTGGTTTACCCCATGG	TICCAAGIGCINGITIACCCCAIGG	TGTGATATGGAGGTGTAGAAGGTGTTA	TCCCAATCTAACTTCCACATACCATCT	TGATCCTGAATGITTALATCITTAACGC	TGGATAGACGTCATATGAAGGTGTGCT	TATHCHTCGTTACTCATGCCATACA	TAACCACCCCAAGATTTATCTTTTGCC A	TeachTechaTechcea	TRATRIDCRIPTROBGIRT	TGCGCGAGCTTTTATTTGGGTTTC	TATITUGGITTCATICCACICAGAICS	TCCTCTTTCACAGGCTCTACTTCATC	TACATCGTTTCGCCCAAGATCAATCA	TTCAAAATGCGGAGGCGTATGTG	TGCCCAGGTACAACCTGCAT	
CAFL_AF053947_33755_33782_R	INV U22457 571 598 R	INV U22457 753 776 R	INV U22457 942 966 R	INV U22457 1619 1643 R	LL NC003143_2367073_23670 97_R	LL NC003143_2367249_23672 71_R	RPLB EC 739 762 TMOD R	RPLB EC 739 762 TWOD R	MECIA Y14051 3367 3393 R	MECA Y14051 3828 3854 R	MECA Y14051 3690 3719 R	MECA Y14051 4555 4581 R	MECA Y14051 4586 4610 R	MECA Y14051 4765 4793 R	MECA Y14051 4590 4600P R	MECA Y14051 4600 4610P R	TRPE AY094355 1569 1592 R	TRPE AY094355 1551 1580 R	TRPE AY094355 1392 1418 R	TRPE AY094355 1171 1196 R	TRPE AY094355 769 791 R	TRPE AY094355 864 883 R	73
286	573	525	664	597	627	550	620	646	653	144	434	288	626	262	389	389	36	557	247	357	135	483	
CAPI_AF053947_33687 TCAGGATGGAAATAACCACCAATTCA 33716 F CTAC	TEGCTCCTTEGTATGACTCTECTTC	TGCTGAGGCCTGGACCGATTATTTAC	TTRITTACCTGCACTCCCACAACTG	TOGTANCHGAGOCTTATAGGOGCA	TETAGCCGCTAAGCACTACCATCC	TEGROSCATORCGATTCTCTAC	TGICCIACIGTIIGIGGTTCTGTAAT	TpCpCpTpTpGITpGICCIACIGTII	TTACACATATCGTGAGCAATGAACTG	TAAAACHAACTACGGTAACATYGATC	TGAAGTAGAATGACTGAACGTCCGA	TCAGGTACTGCTATCCACCCTCAA	TGTACTGCTATCCACCTCAA	TCACCAGGITCAACTCAAAAATAIT	TOPCOACOCOCOCOAA	TCpCpACpCpTpCpAA	ATGTCGATTGCAATCCGTACTTGTG	TOGNITGGCATGGIGANATGGATATGT C	TCAAATGTACAAGGTGAAGTGCGTGA	TOGACOTTINGCAGGAACTAGAC	Dana Tong at an and the same	TGCAAGGGGACCACATACG	
33716 F	INV_U22457_515_539_	INV_U22457_699_724_	INV_U22457_834_858_	INV_U22457_1558_158	1L NC003143 2366996	LL NC003143 2367172	2 614 674 PE	642 679	MECIA Y14051 3315 3	NECA_Y14051_3774_38	MECA Y14051 3645 36	MECA Y14051 4507 45	MECA_Y14051_4510_45	MECA_Y14051_4669_46	MECA V14051 4520 45	MECA_Y14051_4520_45	TRPE_AY094355_1467_	TRPE AY094355 1445	TRPE AY094355 1278	TRPE AY094355 1064	TRPE_AY094355_666_6	TRPE AY094355 757 7	
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	TTCAAGTGCTTGCTCACCATTGTC	TGGCTCATAAGACGCGCTTGTAGA	TTCGGTATAACGCATCGCAGCA	GGIRTRACGCATCGCAGCARAGAITTA	TOSCICASCIATIONCIALIMICOS	TTCCCCTGACCTTCGATTAAAGGATAGC	GAGCTGCGCCAACGAATAAATCGTC	TGCCGTAACATAGAAGTTACCGTTGATT	TACGTCGCCTTTAACTTCGTTATATTCA GC	TCGGGCGTAGTTTTTAGTAATTAAATCA GAAGT	TOGTOGTATTTATAGTGACCAGCACCTA	TTTAAGCGCCAGAAAGCACCAAC	TCAACACCAGOGTTACCTAAAGTACCTT	TCGTTTAAGCGCCAGAAAGCACCAA	TAAGCCAGCAAGAGCTGTATAGTTCCA	TACAGGAGCAGCAGGCTTCAAG	TCGBACCGARGTTACCCTGACCAT	TGCCAGCTTAGTCATACGGACTTC	TATTGCGGATCACCATGATGATATTCTT GC	TOSTIGAGAIGGITTITACCITCGITG	TITGIGAAACAGGAACATTITCITGGI A	TCACGCGCATCATCACCAGTCA	ACCTGCAATATCTAATGCACTCTTACG
	RECA AF251469 140 163 R	RECA AF251469 277 300 R	PARC X95819 201 222 R	PARC X95819 192 219 R	PARC X95819 232 260 R	PARC X95819 143 170 R	OMPA AY485227 364 388 R	OMPA AY485227 492 519 R	ONPA AY485227 424 453 R	OMPA AY485227 514 546 R	OMPA AY485227 569 596 R	OMPA AY485227 658 680 R	OMPA AY485227 635 662 R	OMPA AY485227 659 683 R	OMPA AY485227 739 765 R	OMPA AY485227 786 807 R	GYRA AF100557 119 142 R	GYRA AF100557 178 201 R	GYRB AB008700 111 140 R	GYRB AB008700 369 395 R	GYRB AB008700 466 494 R	GYRR ARDO8700 611 632 R	GYRB AB008700 862 888 R
	109	446	609	609	120	277	655	509	178	206	252	257	328	379	645	613	402	316	289	420	251	179	
	TOGTACATGRECCTICATTGARGCTG	TGACATGCTTGTCCGTTCAGGC	TGGTGACTCGGCATGTTATGAAGC	TGGTGACTCGGCATGTTATGAAGC	Tetroporter	TCAGCGCGTACAGTGGGTGAT	TTACTCCATTATTGCTTGGTTACACT	TOCOLOGICALINGSTRATIONS	DETINED A GREEN CONTRACTOR OF THE PROPERTY OF	TGCCTCGAAGCTGAATATAACCAAGT	TCAACGGTAACTTCTATGTTACTTCT	TCAAGCCGTACGTATTATTAGGTGCT	TCCGTACGTATTAGGTGCTGGTC	TOGTACGTATTATTAGGTGCTGGTCA	TOWNSONGCTTTOTIGGCGCTTAA	TOGISCITTCTQGCGCTTAAACGA	TCTGCCCGTGTCGTTGGTGA	TOTAL THE THE TANKE OF THE TANKE	TO BE CONTROL OF CONTROL	TCTTTCTTGAATGCTGGTGTACGTAT	TCAACGAAGGTAAAAACCATCTCAAC	TGTTCGCTGTTTCACAACAACATC	CA F CARD 760 7 TACTTACTTGAGAATCCACAAGCTGC
76_F	RECA_AF251469_43_68	RECA AF251469 169 1	PARC_X95819_87_110_	PARC_X95819_87_110_	PARC_X95819_123_147	DAPC 294819 43 63 F	OMPA AY485227 272 3	OMPA_AY485227_379_4	OMPA_AY485227_311_3	OMPA_AY485227_415_4	OMPA_AY485227_494_5	ONDA_AY485227_551_5	ONDA AY485227 555 5	OMPA AY485227 556_5	ONPA_AY485227_657_6	OMPA_AY485227_660_6	GYRA_AF100557_4_23_	GYRA_AF100557_70_94	GYRB AB008700 19 40	GYRB AB008700_265_2	92 F GYRB AB008700 368 3	GYRB AB008700 477 5	ON F 0008700 760 7
	806	808	910	- 15		216	2	170	are.	AT6	716	816	616	026	196	200	200	100	976	926	927	876	929

DOCKET NO.: DIBIS-0083USI (COUNSEL DOCKET NO: 10593)

	758	1274	1326	1327	1271	937	1050	1243	1004	905	1362	1399	1104	1090	1384	1363	1170	1126	1379	955	1409	1437	951	1029	1022	1068
	CAAGCGGTTTGCCTCAAATAGTCA	TEGCACGAGCCTGACCTGT	TGTCCGACTTGACGGTCAGCATTTCCTG	TGTCCGACTTGACGGTTAGCATTTCCTG	TGGATGTGCTCACGAGTCTGTGGCAT	TAIGTGCICACGAGITTGCGGCAI	TCCTGCAATATCTAATGCACTCTTACG	TGCTAGACCTTTAGGTGCACCGTG	TCCAGCAGGTTCTGACGGAAACG	TACTAGACGACGGGTCAGGTAACC	TTACCGAGCAGGTTCTGACGGAAACG	TICACCITICCATCITCCAGCCCAT	TOGTOGCGGACTTCGAAGCC	TCGGCATCACGCCGTCGTC	TTOGGGCATCCAGGAGAAGTACATGTT	TIROGCCRICAGGCCRCGCR	TGAGCGTGTGGAAAAGGACTTGGATG	TCICTTICAAGCACCATIGCICATIAT AGI	TTCATTTTCTGGTCCAAAGTAAGCAGTA TC	TCAACTGGTTCAAAACATTAAGTTGTA ATTGTCC	TEGCEGCCATAGCAAAGCCTACAGC	TTIGCTCATGATCTGCATGAAGCATAAA	TCARAGRACCCGCACCTAATTCATCATT TA	TCCCTTAITTTTCFFTCTACTACCTTCG	TCCCCTCATGTTTAAATGATCAGGATAA AAAGC	TCGATCCGCATCACCATCAAAAGCAAA
	WARA 296925 115 138 R	WANA 296925 394 412 R	RPOB EC 3862 3889 R	RPOB EC 3862 3889 2 R	TUFB BC 337 362 R	TUPB EC 337 360 R	GYRB AB008700 862 888 2 R	RFOC EC 2329 2352 R	RPOC EC 1009 1031 R	RFOC EC 2380 2403 R	RFOC EC 1009 1034 R	RFOB EC 2041 2064 R	RPOB EC 1630 1649 R	INFB EC 1414 1432 R	VALS EC 1231 1257 R	RFOC BC 2228 2247 R	CJST CJ 1774 1799 R	CJST CJ 2283 2313 R	CUST CU 663 692 R	CJST CJ 442 476 R	CJST CJ 2753 2777 R	CUST CJ 1406 1433 R	CUST CJ 3356 3385 R	CUST CU 104 137 R	CJST CJ 1166 1198 R	CJST CJ 2148 2174 R
	416	360	581	281	468	493	198	605	404	523	242	387	282	515	237	285	522	388	315	346	504	575	707	222	681	323
на	TCTTGCTCTTTCGTGAGTTCAGTAAA TG	TOGATCTOSTTTCATGCTGTTTCAGT	TGGGCAGCGTTTCGGCGAAATGGA	TGGGCAGCGTTTCGGCGAAATGGA	TGATCACTGGTGCTGCTCAGATGGA	TGCACGCCGACTATGTTAAGAACATG	TACTTACTTGAGAATCCACAAGCTGC AA	TGGTATGTGGTGTGGGC	TCTGGATAACGGTCGTCGCGG	TGCTCGTAAGGGTCTGGCGGATAC	TATTGGACAACGGTCGTCGCGG	DECEDERACACACEATGACGC	TCAGCTGTCGCAGTTCATGGACC	TGCGTTTACCGCAATGCGTGC	TATGCTGACCGACCAGTGGTACGT	TCAGGAGTCGTTCAACTCGATCTACA TGATG	TTAGAGTGATTGACTTTGCTAAAT	TCGTTTGGTGGTGGTAGATGAAAAG	TCCAGGACAATGTATGAAAAATGTC CAAGAAG	TCCTGTTATCCCTGAAGTAGTTAATC	TGCCTAGAAGATCTTAAAAATTTCCG CCAACTT	TGGCTTATCCAAATTTAGATCGTGGT TTTAC	TITGATITIAGGCCGTCCTCCAGGTC	TAGGCGAAGATATACAAAGAGTATTA	TIGAGGIAIGCACCGICTITITAAT	TCCCGGACTTAATATCAATGAAAATT
87_8	WAAA 296925 2 29 F	WAAA_Z96925_286_311 P	RPOB EC 3798 3821 F	RPOB EC 3798 3821 F	TUFB EC 275 299 F	TURB EC 251 278 F	GYRB_AB008700_760_7 87_F	RFOC EC 2223 2243 F	RFOC EC 918 938 F	RFOC EC 2334 2357 F	REOC EC 917 938 F	RPOB EC 2005 2027 P	RPOB EC 1527 1549 F	INFB EC 1347 1367 F	VALS EC 1128 1151 F	RPOC EC 2145 2175 F	CUST CU 1668 1700 F	CUST CU 2171 2197 F	CUST CU 584 616 F	CUST CJ 360 394 F	CUST CJ 2636 2668 F	CUST CJ 1290 1320 F	CUST CU 3267 3293 F	CUST CU 5 39 F	CJST CJ 1080 1110 F	CUST CJ 2060 2090 F
_	931	932	939	940	941	942	949	928	959	960	196	962	963	964	965	978	1045	1046	1047	1048	1049	1050	1051	1052	1053	1054

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TCCTCCTTGTGCCTCAAAACGCATTTTT A	TEGITICITACITICITITECATABACITIT CCA	TGAATTCTTTCAAAGCACCATTGCTCAT TATAGT	TGCAATGTGTGCTATGTCAGCAAAAAGA T	TCCACACAGGATTGTAATTTACCTTGTT CTTT	TCCCGRACAATGAGTTGTATCRACTATT TTTAC	Tacaactgoticaaaacattaagcigt aattgic	TGTGCTTTTTTTGCTGCCATAGCAAAGC	TCGGTTTRAGCICTACATGATCGTARGG	TAIGIGIAGIIGAGCITACTACATGAGC	TRANAGERATTTTACATTTTCG	TCCGATAAGCCGGATTCTGTGC	TGCCGATAAGCCGGATTCTGTGC	TCGITTCACCCTGTCATGCCG	TCGCAGGCTTACAGAACGCTCTCCTA	TOGGACTOGCTTTCGCTACG	TGCTCTTACCTCACCGTTCCACCCTTAC	TITACCICGCCTITCCACCCTTACC	TRAGATITITICCACGGCCGCATC	TAGGATTTTTCCACGGCGCATC	TAGCCTTTTCTCCGGCGTAGATCT	TRANCETCCGATACCAATGGTTCGCTC	TCRACACCTCCTTATTCCCACTC	TCAAGCGATCTACCCGCATTACAA
CJST CJ 2979 3007 R	z.			CJST CJ 2247 2278 R	CUST CJ 711 743 R	CUST CJ 443 477 R	CJST CJ 2760 2787 R	CUST CJ 1349 1379 R	CUST CJ 1795 1822 R	CUST CJ 2965 2998 R	RNPSEP BKM 665 686 R	RNASED BKM 665 687 R	RNASEP BDP 616 635 R	23S BRM 1176 1201 R	23S BRM 616 635 R	RNASEP CLB 498 526 R	RNASEP CLB 498 522 R	ICD CXB 172 194 R	ICD CXB 172 194 R	ICD CXB 224 247 R	IS1111A_NC002971_6928_695 4 R	ISIIIIA_NC002971_7529_755 4 R	REASEP REP 542 565 R
432	31.7	208	660	15	424	345	321	29	479	565	512	333	561	510	496	162	162	343	671	369	290	594	599
TGAAGCITGTTCTTTAGCAGGACTTC	TCCCAATTAATTCTGCCATTTTTCCA	TEGRITGANAAGGGCGAAGTGGCTAAT	Trancetrieseadcinerectian	TGCGGATCGTTTGGTGGTTGTAGATG	TOPASATOTCCANGRAGCATAGCAA	TCCTGTTATCCCTGAAGTAGTTAATC	TCCCCAGGACACCCTGAAATTTCAAC	AGTTATAAACAGGCTTTCCTATGGC TTATCC	TGATTITGCTAAATTTAGAGAAATTG	IGGCALTICITATIGAAGCITGITCIT	TGCGGGTAGGGAGCTTGAGC	TCCTAGAGGAATGGCTGCCACG	TRACTACRACCATCTCCGTG	TGCGCGGAAGATGTAACGGG	TGCATACAAACAGTCGGAGCCT	TANGGATAGTGCAACAGAGATATACC	TAAGGATAGTGCAACAGAGATATACC	TCCTGACCGACCCATTAFTCCCTTTA	TTCCTGACCGACCCATTATTCCCTTT	TOGOGREGABARTCCTACGCT	TCAGTATISTATICACCAGTC	TGGGTGACATTCATCATTTCATCGT	TOGTAAGAGCGCACCGGTAAGTTGGT
	2 2889 2899 E	1 0 TOTAL DOOR TO	2 2212 Z 2012 Z	10 Te43 Te /0 E		COST CO 355 ES	CIST CJ 2678 2703 F	CJST CJ 1268 1299 F	F121 0891 TO TELE	CIST CJ 2857 2887 F	RNASEP BKM 580 599	RNASEP_SKM_616_637_	RNASEP BDP 574 592	23S BRM 1110 1129 F	225 BDM 515 536 F	EP CLB	RMASEP CLB 459 487	TCD CXB 93 120 F	OVE 02 120	TOD CAR 125 158 B	IS1111A MC002971 68	ISI111A NC002971 74	RNASEP_RKP_419_448
_	+	+	1	+	+	0001	1062	1063	590	300	0201	1071	CEO	1073	2020	#/OT	1076	1077	0.00	1010	6,01	1000	1082

957	957	1119	957	1051	910	1310	1147	1305	1415	1147	1330	1230	1226	1324	1163	1370	1164	1009	973	915	1136	1055	1441
TCAAGCGATCTACCCGCATTACAA	TCAAGCGATCTACCCGCATTACAA	TCTATAGAGTCCGGACTTTCCTCGTGA	TCAAGCGATCTACCCGCATTACAA	TCCTGCAGCTCTACCTGCTCCATTA	TAGCAGCAAAAGTTATCACACCTGCAGT	TGGTTGTAGTTCCTGTAGTTGTTGCATT	TGAACATTTGCGACGGTATACCCAT	TGGTGGGTATCTTAGCAATCATTCTAAT AGC	TTGGCGACGGTATACCCATAGCTTTATA	TGAACATTTGCGACGGTATACCCAT	TGTGAACATTTGGGACGGTATACCCAT	TGCGATGGTAGGTATCTTAGCAATCATT CT	TGCCTAACAAATCCCGTCTGAGTTC	TOTCATCAAGCACCCCAAAATGAACT	TGACTITICCICCCCTIATCAGTCICC	TICAMACCITGCTCTCGCCAAACAA	TGAGATGTCGAAAAAAAGGTTGGCAAAA TAC	TCCATATTGTTGCATAAAACCTGTTGGC	TCACCTACAGCTTTAAAGCCAGCAAAAT G	TAGCCTTGGCAACATCAGCAAACT	TCTTCTGTAAAGGGTGGTTTATTATTCA TCCCA	TCCTTCTGATGCCTGATGGACCAGGAG	The state of the s
RNASEP RKP 542 565 R	RNASEP RKP 542 565 R	RNASEP RKP 295 321 R	RNASEP RKP 542 565 R	OMPB RKP 972 996 R	OMPB RKP 1288 1315 R	OMPB RKP 3520 3550 R	GLTA RKP 1138 1162 R	GLTA RKP 499 529 R	GLTA RKP 1129 1156 R	GLTA RKP 1138 1162 R	GLTA RKP 1138 1164 R	GLTA RKP 505 534 R	CTXA VBC 194 218 R	CTXA VBC 441 466 R	RNASEP VBC 388 414 R	TOXR VBC 221 246 R	ASD FRT 86 116 R	ASD FRT 129 156 R	GALE FRT 241 269 R	GALE PRT 901 925 R	GALE PRT 390 422 R	IPAH SGF 301 327 R	4 50 5 000 000
159	310	391	497	654	392	485	576	413	330	553	543	413	410	630	325	362	069	295	658	245	306	458	o in c
TAAGAGCGCACCGGTAAGTTGG	TCCACCAAGAGCAAGATCAAATAGGC	TCINAATGGTCGTGCAGTTGCGTG	TGCATACCGGTAAGTTGGCAACA	TTACAGGAAGTTTAGGTGGTAATCTA	TCTACTGATTTTGGTAATCTTGCAGC ACAG	TGCAAGTGGTACTTCAACATGGGG	TGGGACTTGAAGCTATCGCTCTTAAA GAIG	TCTTCTCATCCTATGGCTATTATGCT	TCOSTTCTTACAATAGCAATAGAAC TTGAAGC	TOGRIGOTIVERAGOTATOGOTOTIVARA	TEGNACTIGNAGCICTOSCICTIANA GAIG	TCTTCTCATCCTATGGCTATTATGCT	TCTTATGCCAAGAGGACAGAGTGAGT	TGTATTAGGGCATACAGTCCTCATC	TCCGCGGAGTTGACTGGGT	TCGATTAGGCAGCAACGAAAGCCG	TIGCTIAAAGIIGGIITTIATIGGIIG	TCAGITITIAATGICICGIATGATCGA AICAAAAG	TIATCAGCTAGACCTTTTAGGTAAAG	TCAAAAGCCCTAGGTAAAGAGATTC CATATC	TCCAAGGTACACTAAACTTACTTGAG CTAATG	TGAGGACCGTGTCGCGCTCA	Camadoo mumo o con a compo a c
RNASEP_RKP_422_443_	RNASEP_RKP_466_491_	RNASEP_RKP_264_287_	RWASEP RKP 426 448	OMPB RKP 860 890 F	CMPB_RKP_1192_1221_ F	OMPB_RKP_3417_3440_	GLTA_RKP_1043_1072_	GLTA RKP 400 428 F	GLTA_RKP_1023_1055_	GLTA_RKP_1043_1072_ 2 F	GLTA_RKP_1043_1072_ 3 F	GLTA RKP 400 428 F	CIXA VBC 117 142 F	CTXA VBC 351 377 F	RNASEP VBC 331 349	TOXR VBC 135 158 F	ASD FRT 1 29 F	ASD FRT 43 76 F	GALE FRT 168 199 F	GALE FRT 834 865 F	GALE FRT 308 339 F	IPAH SGF 258 277 F	
1083	1084	1085	1086	1087	1088	1089	1090	1091	1092	1093	1094	1095	1096	1097	1098	1099	1100	1101	1102	1103	1104	1105	20.0

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TGTCACTCCCGACACGCCA	TOCTCOCOCIACCTACCOG	Camming	TCTCTTACCCCACCCTTTCACCCCTTAC	TAGC	TCCCTAATAGTAGAAATAACTGCATCAG	TOGGCTGTTTTGAGCAACT	TIGHACATTIGAAACAATAIGCAIGACA	TGTGAAT	TCACAGGIICTACTICATCAATAATTIC	TIGCAAICGACATATCCATTTCACCAIG	8	TCCGCCRABARCTCCCCTTTTCACAGG	THE STATE OF THE S	TACGITCTACGATITCTTCATCAGGTAC	ATC	TACAACGTGATAAACACGACCAGAAGC	TAATGCCGGGTAGTGCAATCCATTCTTC	TAG	TGCACCTGCGGTCGAGCG	BO 45 TO 45	TGCCATCCATARTCACGCCATACTGCC	TGCCAGTTTCCACATTTCACGTTCGTG	TOGCTTGAGTGTAGTCATGATTGCG	TOGCTTGAGTGTAGTCATGATTGCG	Comment	TOGCTIGAGIGIAGICALGALIGOS	TGAGTCGGGTTCACTTTACCTGGCA	TGAGTCGGGTTCACTTTACCTGGCA	TACOGGAAGCACCAGCGACATTAATAG
IPAH SGF 522 540 R	RWASEP BRM 542 561 R		RNASEP BRM 402 428 R	HUPB CJ 157 188 R	HUPB CJ 157 188 R		HUPB CU 114 135 K	OIF007 169 203 R	AB MIST-11-	١.	OIF007 364 393 R	AB MLST-11- OIF007 318 344 R	AB MLST-11-	20 VICT-11-	OIF007 656 686 R	AB MLST-11-	AB MIST-11-	OIF007 1266 1296 R	AB MLST-11- OIF007 1299 1316 R	AB MLST-11-	OIP007 1335 1362 R	AB MLST-11- OIF007 1422 1448 R	AB MLST-11- OIF007 1470 1494 R	AB_MLST-11-	AB MLST-11-	OIF007 1470 1494 R	OIF007 1656 1680 R	AB MLST-11- OIF007 1656 1680 R	AB MIST-11-
271	147		185	230	324		324	454		243	541	436		378	250		250	384	384		694	225	383	6,0	200	422	194	684	
THE RESERVED CONTRACTOR OF THE PERSON OF THE	TANACCCCATCGGGAGCAGGCGAA	TA	TACCCCAGGGAAAGTGCCACAGA	TORRESTORY	TCCCGGAGCTTTTATGACTAAAGCAG	TCCCGGAGCTTTTATGACTAAAGCAG	AT	TGAGATTGCTGAACATTTAATGCTGA	TATTGTTTCAAATGTACAAGGTGAAG	TGCG	TEGRACETALCAGETOCCCCASA	TGAAGTGCGTGATGATATCGATGCAC	TOGGITTAGEARAGAAGGIATIGCI	CAACC	Territoria engocamento a some	TCAAGCAGAAGCTTTGGAAGAAGAAG	D.	TOSTGCCOGCAATTIGCATAAAGC	Costemano	TCGTGCCCGCAATTTGCATAAAGC	GRAAC	TAGGTTTACOTCAGTATGGCGTGATT	Agricon a grant and a grant a grant and a	1001001	TTATGGATGGCAACGTGAAACGCGT	TCTTTGCCATTGAAGATGACTTAAGC	TACTAGCGGTAAGCTTAAACAAGATT	TIGCCAATGATATTCGTTGGTTAGCA	TOGGOGAAATOOGTATTCCTGAAAAT
-	ANH SGF 462 486 F	E.	RMASEP BRM 325 347	The same of the sa		HUPB CU 76 102 F	HUPB CJ 76 102 F		OIF007 62 91 F	OIF007 185 214 F	AB MLST-11-		NEW MICH. 11.	OIF007 522 552 F	AB MLST-11-	AB MLST-11-	OLF007 601 627 F	AB_MLST-11-		OIF007 1202 1225 F	OIF007 1234 1264 F	AB MIST-11-	AB MEST-11-	AB MLST-11-	OIF007 1351 1375 F	AB MEST-11-	AB MLST-11-	736	AB MLST-11-
-	+	1111	1112		1128	1129	1130		1151	1152		1133	1154	1155		1156	1157	11.60	200	1159	1160		1441	1162	1163	1164		1165	1166

1195	1151	1224	1157	1228	1039	1037	974	086	1046	1011	1003	1042	1262	1054	1283	1172	1254	1033	1094	806	1291	911
TGCAACTGAATAGATTGCAGTAAGTTAT AAGC	TCAATTATGCAAGAAGTGATCAATTTTC TCACGA	TGCCGTAACTAACATAAGAGAATTATGC AAGAA	TGACGGCATCGATACCACCGTC	TGCCTCGTGCAACCCACCCG	TCCGGCTAGAGATTCTGTATACGACAAT ATC	TOCGCCTTCAAAATGGTGGCGAGT	TCACGATACCTGCATCATCAAATTGGTT	TCAGAATCGATGCCAAATGCGTCATC	TCCICIAIGCAACTIAGIAICAACAGGA AT	TCCATCGCAGTCACGTTTACTGTTGG	TCCACCACCTCAAAGACCATGTGTG	TCGGTCATCGCTGACAGAACTGAGTT	TGGAAACCGGCTAAGTGAGTACCACCAT	TCCTTCACGCGCATCATCACC	TGGCTTGAGAATTTAGGATCCGGCAC	TGAGTCACCCCCCCACAATGTATAGFTCA	TGCTTCAGCACGGCCACCAACTTCTAG	TOCGAGACCAGOSTAGGTGTAACG	TOGGTCAGCAAAACGGTAGCTTGC	TAGAGAGIAGCCATCTTCACCGTTGTC	TOGGGTAAGACGCGGCTAGCATGTATT	TAGCAGCTAGCTOGTAACCAGTGTA
AB_MLST-11- OIF007_1790_1821_R	AB_MLST-11- OIF007_1876_1909_R	AB_MLST-11- OIF007 1895 1927 R	AB MLST-11- OIF007 2097 2118 R	RNASEP BRM 542 561 2 R	CTXB NC002505 132 162 R	FUR WC002505 205 228 R	FUR NC002505 178 205 R	GAPA NC002505 646 671 R	GAPA NC002505 769 798 R	GAPA NC002505 856 881 R	GYRB NC002505_109_134_R	GYRB NC002505 199 225 R	GYRB NC002505 832 860 R	GYRB NC002505 937 957 R	GYRB NC002505 982 1007 R	GYRB NC002505 1255 1284 R	OMPU NC002505 154 180 R	OMPU NC002505 346 369 R	OMPU NC002505 544 567 R	OMPU NC002505 625 651 R	OMPU NC002505 725 751 R	OMPU NC002505 811 835 R
182	656	656	618	147	278	465	465	356	259	517	201	460	236	603	377	148	190	451	266	223	224	181
TACCACTATTAATGTCGCTGGTGCTT	TTAIAACTTACTGCAATCTATTCAGT	TTATAACTTACTGCAATCTATTCAGT	TGGTTATGTACCAAATACTTTGTCTG	TAAACCCCATCGGGAGCAAGACCGAA TA	TCAGCGTATGCACATGGAACTCCTC	TGAGTGCCAACATATCAGTGCTGAAG A	TGRGTGCCAACATATCAGTGCTGAAG A	TCGACAACACCATTATCTATGGTGTG	TCARIGAACGACCACAGAGTGATIGA TG	TGCIAGTCAATCTATCATTCCGGTTG	TGCCGGACAATTACGATTCATCGAGT	TGAGGTGGTGGATAACTCAATTGATG AAGC	TATGCAGTGGAACGATGGTTTCCAAG	TGGTACTCACTTAGCGGGTTTCCG	TCGGGTGATGATGCGCGTGAAGG	TARAGCCCGTGRAATGACTCGTCGTA	TACGCTGACGGAATCAACCAAAGCGG	TGACGGCCTATACGGTGTTTGTTTCT	TCACCGATATCATGGCFTACCACGG	TAGGOGTGAAAGCAAGCTACCGTFF	TAGGTGCTCGTTACGCAGATCAAGA	TACATGCTAGCCGCGTCTTAC
AB MLST-11- OIF007 1726 1752 F	AB MLST-11- OIF007 1792 1826 F	AB_MLST-11- OIF007 1792 1826 F	AB MLST-11- OIF007 1970 2002 F	RNASEP BRM 461 488	CTXB_NC002505_46_70 F	FUR_NC002505_87_113	FUR NC002505_87_113	GAPA_NC002505_533_5 60 F	GAPA_NC002505_694_7	GAPA_NC002505_753_7 82 F	GYRB_NC002505_2_32_ F	GYRB_NC002505_123_1 52 F	GYRB_NC002505_768_7	GYRB_NC002505_837_8 60 F	GYRB_NC002505_934_9 56 F	GYRB_NC002505_1161_	OMPU_MC002505_85_11	OMPU_NC002505_258_2 83 F	OMPU_NC002505_431_4 55 F	OMPU_NC002505_533_5 57 F	OMPU_NC002505_689_7	OMPU NC002505 727 7
1168	1169	1170	1111	1172	2000	2003	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017

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	1302	1424	1077	1233	1017	1277	926	1263	1145	1366	1289	1197	1203	856	1353	1331
	TGGTACTTCAACTTCATCCATTATGAAG TC	TIGITIALIGITICCATATGCTACACAC	TCGCCATAGCTAAGTTGTTTATTGTTTC CAT	TGCGCTATCAACGATTTTGACAATATAT GTGA	TCCCATACCTATGGCGATAACTGTCAT	TGGCCACTTTTATCAGCAACCTTACAGT	TAGECTITIGGAACACCGECTTEATTA AAGT	TGGAACACCGTCTTTAATTAAAGTATCT CC	TCTTTTCTTTGCTTAATTTTCCATTTGC GAT	TTACTTCCTTACCACTTTTAGTATCTAA AGCATA	TOGGGACTICCTTACCACTITTAGTATC	TGCAAGGGAAACCTAGAATTACAAACCC T	TOCATAGGGAAGGTAACACCATAGIT	TAACAACGTTACCTTCGCGATCCACTAA	TGTTGTGCCGCAGTCAAATATCTAAATA	TGTGBAGBACTITCAAATCTGTGAATCC
	AGR-I AJ617706 626 655_R	AGR-II NC002745-2079448- 2080879 700 731 R	AGR-II_NC002745-2079448- 2080879_715_745_R	AGR- IV AJ617711 1004 1035 R	AGR-IV AJ617711 309 335 R	BLAZ NC002952 (191382719 14672) 68 68 R	BLAZ_NC002952 (191382719 14672) 68 68 2 R	BLAZ_NC002952(191382719 14672) 68 68_3 R	BLAZ NC002952 (191382719 14672) 68 68 4 R	BLAZ NC002952 (191382719	BLAZ NC002952(191382719	BSA-A_NC003923-1304065-	BSR-A NC003923-1304065- 1303589 253 278 R	BSA-A MC003923-1304065- 1303589 388 415 R	BSA-A NC003923-1304065- 1303589 317 344 R	BSA-B NC003923-1917149-
	579	415	624	909	562	312	494	467	232	487	351	214	32	679	51.9	209
	TOGGATTTTAAAAACATTGGTAACA	TCTTGCAGCAGTTTATTTGATGAACC	TGTACCCGCTGAATTAACGAATTTAT ACGAC	TGGTATTCTATTTGCTGATAATGAC CTCGC	TOGCACTCTTGCCTTTAATATTAGTA	TCCACTIATCGCAAATGGAAAATTAA GCAA	TGCACTTATCGCAAATGGAAAATTAA GCAA	TGATACTICAACGCCTGCTGCTTTC	DITITION ON CHARLES CAROLING CONTROL AND ATT	TGCAATTGCTTTAGTTTTAAGTGCAT	TCCTTGCTTTAGTTTTTAAGTGCATGT	TAGCGAATGTGGCTTTACTTCACAAT	ATCAATITGGTGGCCAAGAACCTGG	THEACTEGGGCACAACACGGAT	CONTRACTOR	TAGCAACAATATATCTGAAGCAGCG
Es.	AGR- I_AJ617706_580_611_ F	AGR-II_NC002745- 2079448- 2080879 620 651 F	AGR-II NC002745- 2079448- 2080879 649 679 F	AGR- IV_AJ617711_931_961	AGR- IV_AJ617711_250_283	BLAZ NC002952 (19138 271914672)_68_68_	BLAZ NC002952 (19138 271914672) _68_68_	BLAZ NC002952 (19138 271914672) 68_68_ 3 F	BLAZ NC002952 (19138 271914672) 68_68_	BLAZ NC002952 (19138	BLAZ NC002952(19138 27. 1914672) 3 34 F	_	Ι.,	1304065-	BSA-A NC003923- 1304065-	BSA-B NC003923-
	2061	2062	2063	2064	2065	2056	2067	2068	900	0200	2071	2072	2073	2074	100	2076

	1138	1267	1148	1174	1167	1143	964	1266	1397	1041	1429	936	926	11.85	953	1196	919
4	TCITCTIGRADABITGTIGTCCCGAAAC	TGGACTAATAACAATGAGCTCATTGTAC TGA	TGANTATGTANIGCAAACCAGICTITGI CAT	TGAGTCTACACTTGGCTTAGGATGAAA	TGAGCATTTTATATCCATCTCCACCAT	TCTTGGCTTAGGATGAAAATATAGTGGT GGTA	TCANTACAGAGTCTACACTTGGCTTAGG AT	TGGACGATATTCACGGTTTACCCACTTA TA	TIGACATTIGCATGCTTCAAGGCCTG	TCCGTAGITITIGCATAATITATGGICTA TTTCAA	TITATGGTCTATITCAATGGCAGITACG AA	TAIGGICTAITICAAIGGCAGITACGA	TCAACTTCTGCCATTAAAAGTAATGCCA	TGATGGTCTATTTCAATGGCAGTTACGA AA	TCAACAATCAGATGATGTCAGACGCAT G	TGCAAGAGCAACCCTAGTGTTCG	TAGGATGAAAGCATTCCGCTGGC
1914156_1011_1039_R	BSA-B NC003923-1917149- 1914156 1109 1136 R	BSA-B MC003923-1917149- 1914156 1323 1353 R	BSA-B NC003923-1917149- 1914156 2186 2216 R	ERMA_NC002952-55890- 56621_487_513_R	BRMA_NC002952-55890- 56621_438_465_R	ERMA_NC002952-55890- 56621_473_504_R	ERME_NC002952-55890- 56621 491 520 R	ERMA_NC002952-55890- 56621 586 615 R	KRMA NC002952-55890- 56621 640 665 R	2778 173 206 R	ERMC NC005908-2004- 2738 160 189 R	ERMC_NC005908-2004- 2738_161_187_R	Z738 425 452 R	ERMC NC005908-2004-	BRMB_Y13500-625- 1362 352 380_R	BRMB_Y13600-625- 1362_415_437_R	ERMB Y13600-625-
	426	300	703	372	21.7	470	480	297	233	900	298	283	168	421	644	536	556
TACT	TGAARAGTATGGAITTTGAACAACTCG TGAATA	TCATTATCATGCGCCAATGAGTGCAG	TITCATCITATIOGAGGACCCGANATC GA	TCGCTATCTTATCGTTGAGAAGGGAT T	TAGCTATCTTATCGTTGAGAAGGGAT TTGC	TGATCGTTGAGAAGGGATTTGCGAAA	NSCABARTCTGCBACGAGCTTTGG	TCATCCTAAGCCAAGTGTAGACTCTG	TATANGTEGGTAAACCGTGAATATCG	TCTGAACATGATAATATCTTTGAAAT	TCATGATAATATCTTTGAAATCGGCT	TCAGGAAAAGGGCATTTTTACCCTTG	TABATCCTCCTCCTACTACCTA	DD G G G G G G G G G G G G G G G G G G	TOTTGGGAGTATTCCTTACCATTTAA	THEREPARED THE	TGGNTATTCACCGAACACTAGGGTTG
1917149-	Day.	Р								ERMC NC005908-2004-	2738 85 116 F ERNC NC005908-2004-	ERMC NC005908-2004-	ERMC_NC005908-2004-	ERMC_NC005908-2004-	ERMB Y13600-625-	ERMB Y13600-625-	BRMB V13600-625-
	2077		2079	2080	2081		2082		2084	2085	2086	2087	0007	4009	2090	2002	2093

DOCKET NO.: DIBIS-0083USI (COUNSEL DOCKET NO: 10593)

989	1261	993	1124	896	1433	1098	1428	1179	1070	1315	861	862	886	1194	1334
TCATCTGTGGTATGGCGGGTAAGTT	tggraractcatgraactraragtgrarg ga	TCATTAGGTAAAATGTCTGGACATGATC CAA	TCTCATGRARAAGGCTCAGGRGATACRA G	TCACACCTOTAAGTGAGAAAAAGGTTGA T	TTTCCGBTGCRACGTBATGAGATTTCA	TOGIANGACCAGCITOGGTACTACTA	TITATGACCAGCTICGGTACTACTAAA	TGATAATGAAGGAAACCTTTTTCACG	TCGATCGTGACTCTCTTATTTTCAGTT	TGTAATTAACCGAAGGTTCTGTAGAAGT ATG	TACCGTTTCCAAAGGTACTGTATTTTG T	TRACCGTTTCCRARGGTACTGTATTTG TTTACC	TCATCTGGTTTAGGATCTGGTTGACT	TGCAACTCATCTGGTTTAGGATCT	Terechogcatcarcratecta
ERMB_Y13600-625- 1362_521_545_R	PVLUK NC003923-1529595- 1531285 775 804 R	PVLUK_NC003923-1529595- 1531285_1095_1125_R	PVLUK_NC003923-1529595- 1531285 950_978_R	PVLUK_NC003923-1529595- 1531285 654 682_R	SA442_WC003923-2538576- 2538831_98_124_R	SA442_NC003923-2538576- 2538831_163_188_R	SA442_NC003923-2538576- 2538831_161_187_R	SA442_NC003923-2538576- 2538831_231_257_R	SEA_MC003923-2052219- 2051456_173_200_R	SEA_NC003923-2052219- 2051456_621_651_R	SEA MC003923-2052219- 2051456 464 492 R	SEA NC003923-2052219- 2051456 459 492 R	SEB NC002758-2135540- 2135140 273 298 R	SEB_NC002758-2135540- 2135140_281_304_R	SEB NC002758-2135540- 2135140 402 402 R
161	456	539	461	373	635	427	395	226	495	156	629	692	702	244	151
TAAGCTGCCAGCGGAATGCTTTC	TGAGCTGCATCAACTGTATTGGATAG	TGGAACAAATAGTCTCTCGGAATTIT	TGAGTACATCCATATTTCTGCCATA	TCGGAATCTGAGTTGCAGTTGTT	TGTCGGTACGATATTCTTCACGA	TGAAATCICATTACGTTGCATCGGAA A	TCTCATTACGTTGCATCGGAAACA	TAGTACCGAAGCTGGTCATACGA	TGCAGGGAACAGCTTTAGGCA	TAACTCTGATGTTTTTGATGGGAAGG T	TGTATGGTGTGTAACGTTACATGAT	TIGIAIGIAIGEAGGIGIAACGITAC AIGA	TITCACATOTAATITICATAITCGCA	TATTICACATOTAATTITGATATTCG	TRACRACTCGCCTTATCBAACGGGAT ATA
ERMB_Y13600-625- 1362 465 487 F	PVLUK NC003923- 1529595- 1531285 688 713 F	PVLUK NC003923- 1529595- 1531285 1039 1068 F	PVLUK NC003923- 1529595- 1531285 908 936 F	DVLUK NC003923- 1529595- 1531285 610 633 F	SA442 NC003923- 2538576- 2538831 11 35 F	2538576- 2538831 98 124 F	SA442 NC003923- 2538576- 2538831 103 126 F	SA442_NC003923- 2538576- 2538831 166 188 F	SEA_NC003923- 2052219- 2051456 115 135 F		SEA NC003923- 2052219- 2051456 382 414 F		SEB_NC002758- 2135540- 2135140 208 237 F	SEB_NC002758- 2135540-	
2094	2095	2096	2097	2098	2099	2100	2101	2102	2016	2104	2105	2106	2107	80	2109

DOCKET NO.: DIBIS-0083US1 (COUNSEL DOCKET NO: 10593)

TTGTATGTGGTGGTGAACTGAGC 696
TTAACATGAAGGAAACCACTTTGATA ATGG
TGGAATAACAAAGAAAGCAAACC S46
TGAGTTTAACAGTTCACCATATGAAA
TGGTATGATGCTGCACCA 604
TGGFGGTGAAATAGATAGGACTGCTT 615
TGGAGGTGTCACTCCACGAA 554
TTGCACAAGGCGCTAFTT 683
TGGATGTTAAGGGTGATTTTCCCGAA 559
TTTACACTACTTTTATTCATTGCCCT AACG 699
IGATCATCCGTGGTATAACGATTTAT 169
TGACATGATAACCGATTGACCGA AGA
TGTTCAAGAGCTAGATCTTCAGGGAA 640
TGTTCAAGAGCTAGATCTTCAGGCA 639
TCTGGAGGCACCCAAATAAAACA 403
TGCTCAACCCGATCCTAAATTAGACG A 520

DOCKET NO.: DIBIS-0083USI (COUNSEL DOCKET NO: 10593)

1260	1329	1187	927	1390	888	606	996	1316	1129	1293	1118	1049	925	984	1312	1221	706
TGCTTTGTAATCTAGTTCCTGAATAGTA ACCA	TGTCTATTGTCGATTGTTACCTGTACAG	TGALTCARATGCAGAACCATCAAACTCG	TAGTGTTGTACCTCCATATAGACATTCA	TICHGAGCTAAATCAGCAGTTGCA	TACCATCTACCCAACATTAGCACCAA	TAGCACCAATCACCCTTTCCTGT	TCACAAGGACCATTATAATCAATGCCAA	TGTACAAGGACCATTATAATCAATGCCA	TCTGGCCCCTCCATACATGTATTTAG	TGGGTAGGTTTTTATCTGTGACGCCTT	TCTAGCGGAACAACAGTTCTGATG	TCCTGAAGATCTAGTTCTTGAATGGTTA CT	TAGTCCTTTCTGAATTTTACCATCAAAG GTAC	TCAGGTATGAAACACGATTAGTCCTTTC T	TGTABABGCAGGGCTATAATAAGGACTC	TGCCCTTTTGTRARAGCAGGGCTAT	TACTTTAAGGGGCTATCTTTACCATGAA
SEG_NC002758-1955100- 1954171_671_702_R	SEG_NC002758-1955100- 1954171_607_635_R	SRG MC002758-1955100- 1954171 735 762 R	SEH NC002953~60024~	SEH_MC002953-60024- 60977 450 473 R	SEH_MC002953-60024- 60977 608 634 R	SEH_NC002953-60024- 60977 594_616_R	SEI_NC002758-1957830- 1956949_419_446_R	SKI_MC002758-1957830- 1956949 420 447 R	SEI MC002758-1957830- 1956949 449 474 R	SEI_NC002758-1957830- 1956949 290 316 R	SEJ AF053140 1381 1404 R	SEJ AF053140 1429 1458 R	SEJ AF053140 1500 1531 R	SEJ AF053140 1521 1549 R	TSST_NC002758-2137564- 2138293_278_305_R	TSST_NC002758-2137564- 2138293_289_313_R	TSST NC002758-2137564- 2138293 448 478 R
548	555	173	682	201	400	677	253	999	471	394	637	211	153	301	619	514	304
TEGRICARTEGECARTCACTTEGGATIT A.CA	TGGAGGITGTTGTATGGTGGT	TACANAGCAGACACTG	THECAACHGCTGATTTAGCTCAGA	TAGAAATCAAGGTGATAGTGGCAATG	TCTGAATGTCTATATGGAGGTACAAC	TTCTGAATGTCTATATGGAGGTACAA CACT	TCAACTCGAATTTTCAACAGGTACCA	TTCAACAGGTACCAATGATTTGATCT CA	TGATCTCAGAATCTAATAATTGGGAC GAA	TCTCAAGGTGATATTGGTGTAGGTAA CTTAA	TOTTOGRACIA CACTGCATGAAAACAA	TAGCATCAGAACTGTTGTTCCGCTAG	TARCCALTCAAGACTAGATCTTCAG	TCATTCAAGAACTAGATCTTCAGGCA AG	TOSTTTAGATAATTCCTTAGGATCTA TGCGT	TGCGTATAAAAAACACAGAGGCAGC A	TCCBBRTAAGTGGGGTTACABATACT GAA
SEG_NC002758- 1955100- 1954171 623 651 F	SEG NC002758- 1955100- 1954171 540 564 F	SEG_MC002758- 1955100- 1954171 694 718 F	SEH NC002953-60024-	SEH NC002953-60024-	SEH NC002953-60024-	SEH_NC002953-60024- 60977 546 575 F	SEI NC002758- 1957830- 1956949 324 349 F	SEI_MC002758- 1957830- 1956949 336 363 F		SEI NC002758- 1957830- 1955949 223 253 F	SEC AF053140 1307 1	SEJ AF053140_1378_1	SBJ AF053140 1431 1	SEJ_AR053140_1434_1	TSST_NC002758- 2137564- 2138293 206 236 F	ISSI NC002758- 2137564- 2138293 232 258 F	7887_NC002758- 2137564- 2138293_382_410_F
2126	2127	128	2128	2130	1816	2132	2133	2134	2135	200	2137	2138	2139	2140	2141	2142	2143

DOCKET NO.: DIBIS-0083USI (COUNSEL DOCKET NO: 10593)

TSST NC002758-					_
ម្ភី ខ	TCTTTTACRARGGGGRAAAAGTTGA CTT	423	TSST_NC002758-2137564- 2138293 347 373 R	TAAGITCCTTCGCTAGIAIGTTGGCTT	874
100	TOGCOGGCAATGCCATTGGATA	898	ARCC MC003923-2725050- 2724595 97 128 R	TCAGTTAAAATGCGAITGATTTCAGTIT CCAA	1175
TGL	tgaatagtgatagaactgtaggcaca atcgt	437	ARCC_MC003923-2725050- 2724595 214 245 R	TCTTCTTCGTATAAAAGGACCAA	1137
TTG	ttggtcctttttatacgaaagaa gttgaa	691	ARCC MC003923-2725050- 2724595 322 353 R	TOOTGITCTAGTATAGATTGAGGTAGTG	1306
EL	TTGGGAATAGAAGGATGGCTCGT	989	AROK MC003923-1674726- 1674277 435 464 R	TCGAATTCAGCTAAATACTTTTCAGCAT CT	1064
TGG	TGGGGCTTTMATATTCCAATTGAAG ATTTTCA	290	AROK MC003923-1674726- 1674277 155 181 R	TACCTICATTAATICGCTTGTTCATCAA	168
TGAT	igatigg <i>chagtiggatagggtataata</i> Cag	474	AROE MC003923-1674726- 1674277 308 335 R	TAAGCAATACCTTTACTTGCACCACCTG	698
TGCACC	TGCACCGGCTATTAAGAATTACTTTG CCAACT	491	GLPF_MC003923-1296927- 1297391_382_414_R	TGCRACAATTAATGCTCCGACAATTAAA GGATT	1193
TGG	TGGATGGGGATTAGCGGTTACAATG	928	GLPF_MC003923-1296927- 1297391_81_108_R	TARAGREACCGCTGGGTTTRAATGTGCA	850
TAGG	TAGCTGGCGCANATTAGGTGT	21.8	GLPF MC003923-1296927- 1297391 323 359 R	TCACCGATAAATAAAATACCTAAAGTTA ATGCCATTG	972
TACTIT	TACTTTTTAAACTAGGGATGCGFT TGAAGC	200	GNK_MC003923-1190906- 1191334_166_197_R	TGATATTGAACTGGTGTACCATAATAGT TGCC	1180
TGA.	TGAAGTAGAAGGTGCAAAGCAAGTTA GA	435	GNK J7C003923-1190906- 1191334 305 333 R	TOGCTCTCTCAAGTGATCTAAACTTGGA G	1082
TCA	TCACCTCCAAGTTTAGATCACTTGAG AGA	268	GNK WC003923-1190906- 1191334 403 432 R	TGGGACGTAATCGTATAATTCATCATT TC	1284
5 5	TCTTGTTTATGCTGGTAAAGCAGATG G	418	PTA_MC003923-628885- 629355_314_345_R	TGGTACACCTGGTTTCGTTTTGATGATT TGTA	1301
GA G	TGAATTAGTTCAATCATTTGTTGAAC GACGT	439	PTA_NC003923-628885- 629355_211_239_R	TGCATTGTACCGANGTAGTTCACATTGT T	1207

1349	1165	1300	1275	1076	1388	766	1013	1277	926	1263	1145	1366	1289	1277	926	1263
TOTICIOGATIGATIGCACAATCACCAA AG	TGAGATGTTGATGATTTACCAGTTCCGA TTG	TGGTACAACATCGTTAGCTTTACCACTT TCACG	TGCCAGCAATAGTFTGACGTACAAATGC	TOGCCAGCTAGCACGATGTCATTTTC	TICGIGCIGGATITIGICCITIGICCT	TCCAACCCAGAACCACATACTTATTCA C	TCCATCTGTTAAACCATCATATACCATG	TGGCCACTITIATCAGCAACCTTACAGT C	TAGTCTTTTGGAACACCGTCTTTAATTA AAGT	TGGRACACCGTCTTTAATTAAAGTATCT CC	TOTITICUTIGCITAATITICCATTIGC GAI	TTACTTCCTTACCACTTTTAGTATCTAA AGCATA	TOGGGGACTICCTTACCACTITIAGIATC TAA	TGGCCACTTTTATCAGCAACCTTACAGT C	TAGICITTIGGAACACCGGICITTAAITA AAGI	TGGAACACCGTCTTTAATTAAAGTATCT CC
FFA_NC003923-628885- 629355_393_422_R	TPI_NC003923-830671- 831072 209 239 R	TPI_NC003923-830671- 831072_97_129_R	TPI_NC003923-830671- 831072_253_286_R	YQI_NC003923-378916- 379431_259_284_R	YQI_NC003923-378916- 379431_120_145_R	YOL NC003923-378916- 379431 193 221 R	YQI NC003923-378916- 379431 364 396 R	BLAZ (19138271914672)_6 55 683 R	BLAZ (19138271914672)_6 28_659_R	BLAZ (19138271914672)_6 22 651 R	BLAZ_(19138271914672)_5 53 583 R	BLAZ (19138271914672)_1 21 154 R	BLAZ (19138271914672)_1 27 157 R	BLAZ NC002952-1913827- 1914672 655 683 R	BLAZ NC002952-1913827- 1914672 628 659 R	HLAZ_NC002952-1913827- 1914672_622_651_R
303	486	318	246	440	175	314	219	312	494	467	232	487	351	312	494	467
TCCAAACCAGGTGTATCAAGAACATC AGG	TGCAAGTTAAGAAAGCTGTTGCAGGT TTAL	TCCCACGRACAGATGAAGAATTAA CAAAAAAG	TCAAACTGGGCAATCGGAACTGGTAA ATC	TGBATTTGCTGCTATGAAAGGTGGCTT	TACAACATATTAAAGAGACGGGT TTGAATCC	TCCAGCACGAATTGCTGCTATGAAAG	TAGCTIGGCGGTATGGAGAATATGTCT	TCCACTIATCGCAAATGGAAAATTAA	TGCACTTATCGCAAATGGAAAATTAA	TGATACTTCAACGCCTGCTGCTTTC	TATACTTCAACGCCTGCTGCTTTC	TGCAATTGCTTTAGTTTTAAGTGCAT	TCCTTGCTTTAGTTTTAAGTGCATGT	TCCACTTATCGCAAATGGAAAATTAA GCAA	TGCACTTATCGCAAATGGAAAATTAA GCAA	TGATACTTCAACGCCTGCTGCTTTC
PTA_MC003923- 628685- 629355 328 356 F	TPI_NC003923- 830671- 831072 131 160 F	TPI NC003923- 830671- 831072 1 34 F	TPI_MC003923- 830671- 831072 199 227 F	YQI NC003923- 378916- 379431 142 167 F	YQI NC003923- 378916- 379431 44 77 F	YQI_NC003923- 378916- 379431 135 160 F	YQI_NC003923- 378916- 379431 275 300 F	BIAZ (19138271914	BLAZ (19138271914 672) 546 575 2 F	BLAZ (19138271914	BLAZ (19138271914	BLAZ (1913827.1914 672) 24 56 F	BLAZ (19138271914 672) 26 58 F	BLAZ NC002952- 1913827-		BLAZ MC002952- 1913827- 1914672 507 531 F
2159	2160	2161	2162	2163	2164	2165	3312	23.67	21.68	21.69	2170	2171	2172	2173	21.74	2175

					_								_	_	
1145	1366	1289	1321	1321	1321	1311	922	1382	899	1354	928	853	979	1107	1102
TCTTTTCTTIGCTTAATTTTCCATTIGC	THCTTCCTTACCACTTTTAGTATCTAA AGCATA	TEGGGACTICCTTACCACTTTAGTATC	TGTCACCAGCTTCAGCGTAGTCTAATAA	TGTCACCAGCTTCAGCGTAGTCTAATAA	TGTCACCAGCTTCAGCGTAGTCTAATAA	TGGTTTGTCAGAATCACGTTCTGGAGTT GG	TAGGCATAACCATTICAGTACCTTCT3G TAA	TICCALTICAACIBATICIBATAATICT TCATCGIC	TACGCTAAGCCACGTCCATATTTATCA	RETITIVERSATIGGENETAGGETA	TAGITGAAGITGCACIATATACTGTIGG A	TAAATGCACTTGCTTCAGGGCCATAT	TCACGTCGACTTCACGGTCAGGAT	TOGIOGGACITAACGGICAGCAITICCI	TOGICOGACTTAACGGTCAGCATTTCCT
BLAZ NC002952-1913827- 1914672 553 583 R	BLAZ MC002952-1913827- 1914672 121 154 R	BLAZ MC002952-1913827- 1914672 127 157 R	TUFB_NC002758-615038- 616222_793_820_R	TUFB_NC002758-615038- 616222_793_820_R	TUFB NC002758-615038- 616222 793 820 R	TUFE_NC002758-615038- 616222_601_630_R	TUFE MC002758-615038- 616222 1030 1060 R	TUFB_NC002758-615038- 616222 424 459 R	NUC_NC002758-894288-894974 483 509 R	NUC_MC002758-894268- 894974_165_189_R	NUC_NC002758-894288- 894974_222_250_R	NUC_NC002758-894288- 894974 396 421 R	BD08 38 3895 R	RPOB RC 3860 3890 R	RPOB EC 3860 3890 2 R
232	487	351	643	386	430	320	433	307	342	349	273	174	935	200	294
PATACTICAAGGCCTGCTGCTTTC	TGCAATTGCTTTAGTTTTAAGTGCAT	TOCTIGOTITAGITITIAAGIGCATGI AAITCAA	TGTTGAACGTGGTCAAATCAAAGTTG GTG	TCSTGTTGAACGTGGTCAAATCAAAG T	TGBACGTGGTCBARTCBARGTTGGTG ABGA	TCCCAGGIGACGAIGTACCTGTAATC	TGAAGGTGGACGTCACACTCCATTCT	TO THE PRODUCTION OF THE PRODUCTION OF THE PROPULTION OF THE PROPULT OF THE PROPU	TOCHION NOT SANDON TOTAL COMME	TCCITATAGGGAIGGCIATCAGTAAT	TCAGCAAATGCATCACAAACAGATAA	TACAAAGGTCAACCAATGACATTCAG	ACIA	TGGCCAGCGCTTCGGTGHIAIGGA	RPOB EC 3789 3812 F TUMSTICAGUSSICAGUSCITUCES PRODU RC 3789 3812 F TUMSTICAGUSGUSGUSGUSGUSGUSGUSGUSGUSGUSGUSGUSGUSG
1913827- 1913827-		. Do			TUFB NC002758- 615038- 616222 696 725 F	TUFB_NC002758- 615038-	TUFB NC002758- 615038-	TUFB MC002758- 615038-	NUC NC002758- 894288-	894974 402 424 F NUC NC002758- 894288-	NUC_NC002758- 894288-	NUC NC002758-	894974 316 345 F RPOB EC 3798 3821 1	Eu .	RPOB EC 3789 3812 F
	\vdash	2177	1400	22 22	2249	0		7727	2252	2253	1,077	2222	2256	2270	2271

DOCKET NO.: DIBIS-0083US1 (COUNSEL DOCKET NO: 10593)

1106	1101	1105	1100	1030	1711	931	1360	1103	981	1127	1317	1109	1109	855	855	1027	855	1154	864	1296
TOGICGGACTTAACGGTCAGCATTICCT G	TOGICCGACITAACGGICAGCATITCCI G	TCGTCGGACTTAACGGTCAGCATTTC	TOGICCGACTTAACGGTCAGCATTTC	TCCCTTCCTTAATATGAGAAGGAAACCA	TOAGCTGGTGCTATATGAACAATACCAG T	TATATGAACAATACCAGTICCTTCTGAG T	THAICIGGCIGCGGAAGIGAAAICGI	TOGICCICGAAICTCCGAIAIACC	TCAGATATAAATGGAACCAATGGAGCCA CT	TOTISCATITITIGGAGCCHGTCTA	TGTACAATAAGGAGTCACCTTATGTCCC	TOGRECTARCHARTCCCGTCTGAGTTC	TCGTGCCTAACAAATCCCGTCTGAGFTC	TAACAAATCCCGTCTGAGTTCCTCTTGC A	TAACAAATCCCGTCTGAGTTCCTCTTGC A	TCCCGTCTGAGTTCA	TAACAAATCCCGTCTGAGTTCCTCTTGC A	TGACCCAAAGCTGAAAGCTTTACTG	TAACTGACCCAAAGCTGAAAGCTTTACT G	TGGGTTGCGTTGCAGAFTATCTTTACCA
RPOB BC 3862 3890 R	RPOB EC 3862 3890 2 R	RPOB BC 3865 3890 R	RPOB EC 3865 3890 2 R	MUPR X75439 1744 1773 R	MUPR X75439 1413 1441 R	MUPR X75439 1381 1409 R	MUPR X75439 2548 2574 R	MUPR X75439 2605 2630 R	MUPR X75439 2711 2740 R	MUPR X75439 2867 2890 R	MUPR X75439 977 1007 R	CTXA_NC002505-1568114- 1567341 194 221 R	CTXA_NC002505-1568114- 1567341 194 221 R	CTXA_NC002505-1568114- 1567341 186 214 R	CTXA_NC002505-1568114- 1567341 186 214 R	CTXA NC002505-1568114- 1567341 180 207 R	CTXA_NC002505-1568114-	INV U22457-74- 3772 942 966 R	INV U22457-74- 3772 942 970 R	INV U22457-74-
294	294	674	674	352	699	704	172	188	513	165	447	809	411	809	411	27	200	530	438	526
TCAGITCGGGGGTCAGCGCITCGG	TCAGITCGGCGGTCAGCGCTTCGG	TTCGGCGGTCAGCGCTTCGG	TTCGGCGGTCAGCGCTTCGG	TCCTTTGATATATATGCGATGGAAG	TTCCTCCTTTTGAAAGCGACGGTT	TITICETCETTITIGAAAGCGACGGIT	TAATTIGGGCTCTTTCTCGCTTAAACA	TACGRITICACTICGCNGCCAGAIT	TGCGTACAATACGCTTTATGAAATTT TAACA	TAATCAAGCATTGGAAGATGAAATGC	TGACATGGACTCCCCTATATAACTC	TOGICITATGCCAAGAGGACAGAGTG	TCTTATGCCAAGAGGACAGAGTGAGT	TGGTCTTATGCCAAGAGGACAGAGTG AGT	TCTTATGCCAAGAGGACAGAGTGAGT ACT	AGGACAGAGTGAGTACTTTCACCGAG GT	TGCCAAGAGACAGAGTGAGTACTTT GA	TGCTTATTTACCTGCACTCCCACAAC TG	TGAATGCTTATTTACCTGCACTCCCA	TGCTGGTAACAGAGCCTTATAGGCGC
RPOB EC 3789 3812 F	RPOB EC 3789 3812 F	RPCB EC 3793 3812 F	RPOB EC 3793 3812 F	MUPR_X75439_1658_16	MUPR_X75439_1330_13	MUPR X75439 1314 13	NUPR_X75439_2486_25 16 F	MUPR_X75439_2547_25 72_F	MUPR_X75439_2666_26 96 F	MUPR_X75439_2813_28	MUPR_X75439_884_914 F	CTXA_NC002505- 1568114- 1567341 114 142 F	CTXA_NC002505- 1568114- 1567341 117 145 F	CIXA_NC002505- 1568114- 1567341 114 142 F		CTXA_NC002505- 1568114- 1567341 129 156 F	CTXA_NC002505- 1568114- 1567341 122 149 F	3772 831 858 F	3772 827 857 F	INV U22457-74-
2273	2274	2275	2276	2309	2310	2312	2313	2314	2315	2316	2317	2318	2319	2320	2321	2322	2323	2324	2325	2326

	1 TOOT COOT 7//0	¥		3772 1619 1647 R	A	
2327	3772 1558 1585 F	TGGTAACAGAGCCTTATAGGGGGCATA TG	598	INV U22457-74-	TCATAAGGGITGCGITGCAGATTATCTT	
2328	ASD_NC006570- 439714- 438608 3 37 F	TGAGGGTTTTATGCTTAAAGTTGGTT	0.74	ASD_NC006570-439714-	TGATTCGATCATACGAGACATTAAAACT	286
2329	ASD NC006570- 439714- 438608 18 45 F	IMAGTIGGILITATIGGITGGCGCG	070	ASD NC006570-439714-	TCAAAATCTTTTGATTCGATCATACGAG	1188
2330	ASD_NC006570- 439714- 438608_17_45_F	TTALAGTIGGITTIATIGGITGGCGC	647	ASD NC006570-439714- 438608 67 95 R	AC TCCCAATCTTTGATTCGATCATACGAG A	1016
2331	ASD_NC006570- 439714- 438608_9_40_F		709	ASD_NC006570-439714- 438608 107 134 R	TCTGCCTGAGATGTCGAAAAAAACCTTG	-
2332	GALE_AF513299_171_2 00_F		280	GALE AF513299 241 271 R	TCTCACCTACAGCTTTAAAGCCAGCAAA	201
2333	GALE_AF513299_168_1 99_F		658	GALE AF513299 245 271 R	になっていりまるのはいいかっているのはいいと	
2334	GALE_AF513299_168_1	TIATCAGCTAGACCTTTTAGGTAAAG	859	GALR AFE13299 233 264 B	TACAGCITTARAGCCAGCAAAATGAATT	1911
2335	GALE_AF513299_169_1	TCCCAGCTAGACCTTTTAGGTAAAGC TAAG	319	GALE AF513299 252 279 B	THICK STORY CHORDS AND ASSESSMENT AND	283
2336	PLA_AF053945_7371_7 403_F	TIGAGAGACAICCGGCICACGITAT TAIGGIA	680	DIA 28053945 7434 7458 D	TACGTATGTAAATTCCGCAAAGACTTTG	13/4
2337	FLA AF053945_7377_7	TGACATCGGGCTCACGTTATTATGGT	443	PLA AP053945 7428 7455 P	TOCOCH AND COMMENCED AND COMMENCED	005
2338	PLA AF053945 7377 7	TGACATCCGGCTCACGTTATTATGGT AC	444	PLA 2F053945 7430 7460 D	TABATTCCCCAAAGACTTTGCCATTAGG	1035
2339	CAF_AF053947_33412_ 33441_F	-	329	CAF AF053947_33498_33523_	To a some post of the part of	100
2340	CAF AF053947_33426_ 33458 F	TGCATTATTTGGAACTATTGCAACTG	499	CAF_AF053947_33483_33507_	Tegricalcalcalcarrectarrecta	1308
2341	CAF AF053947_33407_ 33429 F	TCAGITCCGTTATCGCCATTGCA	291	CAF_AR053947_33483_33504_	TTCAACAAGAGTTGCCGTTGCC	133
2342	33431 F	-	293	CAP_AF053947_33494_33517_	TGATGCGGGCTGGTTCAACAAGAG	1184
2344	GAPA_NC_002505_1_28 F_1	TCAATGAACGATCAACAAGTGATTGA TG	260	GAPA NC 002505 29 58 R 1	TCCITTAIGCAACTIGGIATCAACAGGA	1060
2472	P	TGCCTGTAGGGAATCCTGCTGA	502	OMPA NC000117 145 167 R	TCACACCAAGTAGTGCAAGGATC	196
2473	21 F	-	475	OMPA NC000117 865 893 R	PCMAACTIGCTCTAGACCATTTAACTC C	947
2474	71 F	TGCTCAATCTAAACCTAAAGTCGAAG A	521	OMPA NC000117 757 777 R	TGTCGCACCATCTGTTCCTGC	1 2 2 8
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1153	1371	851	1406	1395	1058	1025	1270	903	1062	1323	1396	1251	1419	1010	1073	1416	1279	1032	873	1236	1951	1234
TGACAGGACACAATCTGCATGAAGTCTG AG	TTCHANAGTTGCTCGAGACCATTG	TARAGAGACGITITGGTAGTICATITGC	TIGCCATICATGENTITAAGIGIAGCA	TICITGAAGGGGGGTTTCGATTG	TCCTTTAAAATAACCGCTAGTAGTAGTCCT	TCCCGCTGGCAAATAAACTCG	TGGATCACTGCTTACGAACTCAGCTTC	TACGITIGIATCITCIGCAGAACC	TCCTTTCAATGTTACAGAAAACTCTACA G	TGTCAGCTAAGCTAATAACGTTTGTAGA G	TTGACATCGTCCCTCTTCACAG	TGCTGTAGGGAAATCAGGGCC	TIGICAGACICATOGOGAACAIC	TOCATACARACCARAGETRACCITG	TOSCAGOGINGCATOGONC	ATRODOTTO ATROUCH	PROCESTIGECATIONS	TCCGAAGTTGCCCTGCCGTC	TAAGTTACCTTGCCCGTCAACCA	TGCGGGTGATACTTACCGAGTAC	OCCUPATION OF THE PROPERTY OF	TECGGCACCTATCACCATCCA
OMPA NC000117 1011 1040 R	OMPA NC000117 871 894 R	OMPA NC000117 511 534 R	OMPA NC000117 787 816 R	OMPA NC000117 649 672 R	OMPA NC000117 417 444 R	OMP2 NC000117 71 91 R	OMP2_NC000117_445_471_R	OMPZ WC000117 1396 1419 R	OMP2 NC000117 1541 1569 R	OMP2 NC000117 120 148 R	OMP2 NC000117 240 261 R	GYRA NC000117 640 660 R	GYRA NC000117 871 893 R	GYRA NC002952 319 345_R	GYEA NC002952 1024 1041 R	GYRA NC002952 1546 1562 R	GYRA NC002952 124 143 R	GYRA NC002952 313 333 R	GYRA MC002952 308 330 R	GYRA MC002952 220 242 R	0 539 543 653 D	GYRA NC002952 338 360 R
157	196	676	212	571	492	234	516	537	407	450	441	287	989	632	176	366	279	452	625	53	100	380
TAACTGCATGGAACCCTTCTTTACTA G	TACTEGRACIANGECTIGGGACC	TICIAICIGITIGGITTATICGGAGT T	TAGCCCAGCACAATTTGTGATTCA	TEGCGINGINGAGCIATITACAGACA	TGCACGATGCGGAATGGTTCACA	TATGACCAAACTCATCAGACGAG	TGCTACGGTAGGATCTCCTTATCCTA	TGGAAAGGTGTTGCAGCTACTCA	TCTGGTCCAACAAAGGAACGATTAC AGG	TGACGATCTTCGCGGTGACTAGT	TGACAGCGAAGAAGGTTAGACTTGTC C	TCAGGCATTGCGGTTGGGATGGC	TGTGAATAAATCACGATTGATTGAGC A	TOTCATGGGTAAATATCACCCTCA	TACAAGCACTCCCAGCTGCA	TCGCCCGCGAGGACGT	TCAGCTACATCGACTATGCGATG	TGACGTCATCGGTAAGTACCACCC	TGTACTCGGTAAGTATCACCCGCA	TGAGATGGATTTAAACCTGTTCACCG	Commence of the second	TOGIATGGCTCAATGGTGGAG
OMEA_NC000117_947_9	OMPA_NC000117_774_7 95 F	OMPA_NC000117_457_4 83 F	OMPA_NC000117_687_7	1	OMPA_NC000117_338_3	OMP2_NC000117_18_40	OMP2_NC000117_354_3 82 F	OMP2_NC000117_1297_ 1319 F	OMP2_NC000117_1465_ 1493 F	OMP2_NC000117_44_66	OMP2_NC000117_166_1 90 F	GYRA_NC000117_514_5 36 F	GYRA_NC000117_801_8 27 F	GYRA NC002952 219 2	GYRA NC002952_964_9 83 F	GYRA NC002952 1505 1520 F	GYRA_NC002952_59_81	GYRA_NC002952_216_2 39 F	GYRA_NC002952_219_2	GYRA_NC002952_115_1	GYRA NC002952 517 5	GYRA NC002952 273 2
2475	2476	2477	2478	2479	2480	2481	2482	2483	2484	2485	2486	2487	2488	2489	2490	2491	2492	2493	2494	2495	0.00	2497

	1067	1116	904	1355	1020	1117	1084	1245	1427	950	1348	1432	966	1205	1257	920	917	1417	942	1443	1065
	TOGAGCOGAAGTTACCCTGTCCGTC	TCpTpTpTpCpGTATAAAAAGGACpCpA ATpTpGG	TACDACDCDIGGTPTPTDCpGTpTpTpT pGATGATpTpTpGTA	TOTTTTATOTOTAGTTGAGCTTACTACA	TCCCCATCTCCGCAAAGACAATAAA	TCTACAACACTTGATTGTAATTTGCCTT	TOGGRANCHAGANITICATITITICIGGTC	TGCTATATGCTACAACTGGTTCAAAAC ATTAAG	TITAGCTACTATTCTAGCTGCCATTTCC	TCAAAGAACCAGCACCTAATTCATCATT	TGTTCCAATAGCAGTTCCGCCCAAATTG AT	TITCCCCGATCTAAATTTGGATAAGCCA TAGGAAA	TOCRARCGRICTGCRICRCCATCRARAG	TGCATGAAGCATAAAAACTGTATCAAGT GCTTTTA	TGCTTGCTCAATCATCATAAAAATTA	TAGGATGAGCATTATCAGGGAAAGAATC	TAGCGATTICTACTCCTAGAGTTGAAAT	TIGGITCITACTIGITITGCATAAACTT TCCA	TAITGCTTTTTTTGCTAIGCTTCTTGGA	TTTIGGTCATGATCTGCATGAAGCATAA A	TOGACCTGGAGGACGACGTAAAATCA
	GYRA NC000912 346 370 R	ARCC_NC003923-2725050- 2724595_214_239P_R	PTA_NC003923-628885- 629355 314 342P R	CUMIST ST1 1945 1977 R	CJMLST ST1 3073 3097 R	CJMLST ST1 2447 2481 R	CUMIST ST1 725 756 R	CJMLST ST1 454 487 R	CJMLST ST1 1312 1340 R	CJMLST ST1 3656 3685 R	CUMIST ST1 55 84 R	CJMLST ST1 1383 1417 R	CUMILST ST1 2352 2379 R	CJMLST ST1 1486 1520 R	CUMIST ST1 3511 3542 R	CUMIST ST1 1203 1230 R	CUMIST ST1 2940 2973 R	CUMIST ST1 2131 2162 R	CUMIST ST1 655 685 R	GLTA_NC002163-1604930- 1604529 352 380 R	UNCA NC002163-112166-
	462	229	417	708	428	535	240	347	564	529	145	538	582	534	692	189	591	241	344	299	322
	TGAGTAAGTTCCACCOGCACGG	TAGTDGATDAGAACPTDGTAGGCDAC DAATDCDGT	TCTTGTpTpTpATGCpTpGGTAAAGC AGATGG	TITIGGGAIGAAGTAGGTGCCTATCT	TGAAATTGCTACAGGCCCTTTAGGAC	TGCTTTTGATGGTGATGCAGATCGTT TGG	TATGTCCAAGAAGCATAGCAAAAAA	TCCTGTTATTCCTGAAGTAGTTAATC	TGGCAGTTTTACAAGGTGCTGTTTCA TC	TGCTGTAGCTTATCGCGAAATGTCTT	TANAMACTITITGCCGTAATGATGGGTG	TGGRAATGGCAGCTAGAATAGTAGCT	TGGGCCTAATGGGCTTAATATCAATG	TGCTTTCCTATGGCTTATCCAAATTT	TTGTAAATGCCGGTGCTTCAGATCC	TACGCGTCTTGAAGCGTTTCGTTATG A	TGGGGCTTTGCTTTATAGTTTTTTAC	TATTCAAGGTGGTGCTCTTGATGCATG T	TCCTGATGCTCAAAGTGCTTTTTTAG	TCATGTTGAGCTTAAACCTATAGAAG TAAAAGC	TCCCCCACGCTTTAATTGTTTATGAT
93_F	GYRA ACCOCOSIZ 257 Z	ARCC_NC003923- 2725050- 2724595_135_161P_F	PTA_NC003923- 628885- 629355 237 263P F	3 F	CUMLST_STI_2963_299	CUMLST ST1 2350 237	COMLST ST1 654 684	CUMLST_ST1_360_395_	CUMEST_STI_1231_125	CUMLST_ST1_3543_357 4 F	CUMIST STI 1 17 F	2_F	CUMLST_ST1_2254_228 6 F	CUMLST_ST1_1380_141	CUMLST ST1 3413 343	CUMIST_STI_1130_115	CJMLST_ST1_2840_287 2 F	CUMLST_ST1_2058_208	CUMLST_STI_553_585_F	GLTA_MC002163- 1604930- 1604529_306_338_F	UNCA NC002163-
	2498	2504	2505	2517	2518	2519	2520	2521	2522	2523	2524	2525	2526	2527	2528	2529	2530	2531	2532	2564	2565

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	TGGGATAACATTGGTTGGAATATAAGCA GAJACATC	TOCATOGCCAGTITITICATAATOGCIA AAAA	TCAAAACGCAITITTACAICTICGITAA AGGCTA	TGTTCATGTTTAAATGATCAGGATAAAA AGCACT	TGCCATAGCAAAGCCTACAGCATT	TACATCTCCTTCGATAGAATTTTCATTG	Tamba Camber tite of Califity and Tiber	TTGCCATAGCAAAGCCTACAGCATT	TGCCATTTCCATGTACTCTTCTCTAACA	ATTECTION TROUBERT ATTECTION OF THE STATE STATES ATTECTS ATTECT	TGCTCACCTGCTACAAGTCCAGCAA	TTCCACCTTGGATACCTGGAAAAATAGC	TCAAGCTCTACACCATAAAAAAAGCTCT	TITIGOCOCCAAAGITITOCAC	TOCCCCATTGCTCATGATAGTAGCTAC
112647_146_171_R	UNCA NC002163-112166-	PGM_NC002163-327773- 328270 365 396 R	TKT_NC002163-1569415- 1569873 350 383 R	GLTA_NC002163-1604930- 1604529_109_142_R	TKT_NC002163-1569415- 1569903_139_162_R	TKT_NC002163-1569415- 1569903 313 345 R	TKT_MC002163-1569415- 1569903 449 481 R	TKT_NC002163-1569415- 1569903 139 163 R	GLTA_NCO02163-1604930- 1604529 139 168 R	GLYA_NC002163-367572- 368079 476 508 R	GLYA_NC002163-367572- 368079 242 270 R	GLVA_MC002163-367572- 368079 384 416 R	GLYA_NC002163-367572- 368079 52 81 R	PGM_MC002163-327746- 328270 356 379 R	PGM_MC002163-327746- 328270_241_267_R
	170	414	661	381	472	164	213	665	382	1881	611	622	614	455	425
GATTICAG	Thatgaattaggigcgggttctt T	TCTTGAFACTTGFAATGTGGGCGATA AATATGF	TTATGAAGCGTGTTCTTFAGCAGGAC	TOSTCTITITISATICITITICCCTGATA AIGC	TGATCTTAAAATTTCCGCCAACTTC ATTC	TAAGGTTTATTGTCT-TYGYGGAGATG GGGATTT	TAGCCTTTRACGAAAATGTAAAAATG CGTTTTGA	TTCARARACTCCAGGCCATCCTGAAA TYTGAAC	TCGTCTTTTGATTCTTTCCCTGATA ATGCTC	TCAGCTATTTTCCAGGTATCCAAGG TGG	IGGIGCSAGIGCTIAIGCICGIAITA T	TGTAAGCTCTACAACCCACAAAACCT TACG	TGGTGGACATTTAACACATGGTGCAA	TGAGCAATGGGGCTTTGAAAGAATTT TTAAAT	TGAAAAGGGTGAAGTAGCAAATGGAG ATAG
112166- 112647 80 113 F	UNCA_NC002163- 112166- 112647_233_259_F	PGM_NC002163- 327773- 328270_273_305_F	TKT_NC002163- 1569415- 1569873_255_284_F	GLTA NC002163- 1604930- 1604529 39 68 F	TKT MC002163- 1569415- 1569903 33 62 F	TKT_NC002163- 1569415- 1569903_207_239_F	TKT_NC002163- 1569415- 1569903 350 383 F	1KT_NC002163- 1569415- 1569903 60 92 F	GLTA_NC002163- 1604930- 1604529_39_70_F	GLYA_NC002163- 367572- 368079_386_414_F	GLYA_NC002163- 367572- 368079 148 174 F	GLYA_NC002163- 367572- 368079_298_327_F	GLYA_NC002163- 367572- 368079 1 27 F	PGM_NC002163- 327746- 328270_254_285_F	PGM_NC002163- 327746-
	2566	2567	2568	2570	2571	2572	2573	2574	2575	2576	2577	2578	2579	2580	2581

	328270_153_182_F					
2582	PGM_NC002163- 327746- 328270 19 50 F	TGGCCTAATGGGCTTAATATGAATGA AAATTG	568	PGM_NC002163-327746- 328270_79_102_R	TGCACGCAAACGCTTTACTTCAGC	1200
2583	UNCA_NC002163- 112166- 112647 114 141 F	TRAGCATGCTGTGGCTTATCGTGRAR TG	160	UNCA_NC002163-112166- 112647_196_225_R	TGCCCTTTCTAAAGTCTTGAGTGAAGA TA	1220
2584	UNCA_NC002163- 112166- 112647 3 29 F	TGCTTCGGATCCAGCAGCACTTCAAT A	532	UNCA NC002163-112166- 112647 88 123 R	TGCATGCTTACTCAAATCATCATAAACA ATTAAAGC	1206
2585	ASPA_NC002163- 96692- 97166 308 335 F	TTRATTTGCCAAAAATGCAACCAGGT AG	652	ASPA_NC002163-96692- 97166_403_432_R	TGCAAAAGTAACGGTTACATCTGCTCCA AT	1192
2586	ASPA_NC002163- 96692- 97166_228_258_F	TCGCGTTGCAACAACTTTCTAAAG TATGT	370	ASPA_NC002163-96692- 97166_316_346_R	TCATGATAGAACTACCTGGTTGCATTTT TGG	991
2587	GENNA_NC002163- 658085- 657609 244 275 F	TGGAATGATGATAAAGATTTCGCAGA TAGCTA	547	GIAR NC002163-658085- 657609_340_371_R	TGAGTTTGAACCATTTCAGAGCGAATAT CTAC	1176
2588	IKT_NC002163- 1569415- 1569903 107 130 F	TOGCTACAGGCCCTTTAGGACAAG	371	TKT_NC002163-1569415- 1569903 212 236 R	TOCCCATCTCCGCAAAGACAATAAA	1020
2589	TKT_NC002163- 1569415- 1569903 265 296 F	TGTTCTTTAGCAGGACTTCACAAACT TGATAA	642	TKT_NC002163-1569415- 1569903 361 393 R	TCCTIGTGCTTCAAAACGCATTTTTACA TTTTC	1057
2590	GLYA_NC002163- 367572- 368095 214 246 ?	TGCCTATCTTTTTGCTGATATAGGAC	2002	GLYA NC002163-367572- 368095 317 340 R	TOCTCTTGGGCCACGCAAAGTTTT	1047
2591	GLYA_NC002163- 367572- 368095 415 444 F	TCCTTTGATGCATGTAATTGCTGCAA	353	GLYA_NC002163-367572- 368095 485 516 R	TCTTGAGCATTGGTTCTTACTTGTTTTG	1141
2592	PGM_NC002163_21_54_	TCCTAATGGACTTAATATCAATGAAA	332	PGM NC002163 116 142 R	TCAAACGATCCGCATCACCATCAAAAG	949
2593	PGM_NC002163_149_17	TAGATGAAAAGGCGAAGTGGCTAAT GG	207	PGM NC002163 247 277 R	TCCCCTTTAAAGCACCATTACTCATTAT AGT	1023
2594	GIMA_NC002163- 658085- 657609 79 106 F	TGTCCAAGAAGCATAGCAAAAAAGC AA	633	GLARA_NC002163-658085- 657609_148_179_R	TCAAAAACAAAGAATTCATTTTCTGGTC CAAA	945
2595	ASPA_NC002163- 96685- 97196 367 402 F	TCCTGTTATTCCTGAAGTAGTTAATC AAGTTTGTTA	347	ASPA_NC002163-96685- 97196_467_497_R	TCAAGCTATATGCTACAACTGGTTCAAA AAC	960
2596	ASPA_NC002163- 96685-97196 1 33 F	TGCCGTAATGATAGGTGAAGATATAC	502	ASPA_NC002163-96685- 97196_95_127_R	TACAACCTTCGGATAATCAGGATGAGAA TTAAT	880
2597	ASPA_NC002163- 96685-	TGGAACAGGAATTAATTCTCATCCTG ATTATCC	540	ASPA NC002163-96685- 97196 185 210 R	TAMBETECEGIATETTGAGTEGECTE	872

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	971.96_85_117_F					
2598	PGM_NC002163- 327746- 328270 165 195 F	TGGCAGCTAGAATAGTAGCTAAAATC CCTAC	563	PGM_NC002163-327746- 328270_230_261_R	TCACGATCTAAATTTGGATAAGCCATAG GAAA	975
2599	PGM_NC002163- 327746- 328270 252 286 F	TGGGTCGTGGTTTTACAGAAATTTC TTATATATG	593	PGM_NC002163-327746- 328270_353_381_R	TTTTGCTCATGATCTGCATGAAGCATAA A	1443
2600	PGM_NC002163- 327746- 328270 1 30 F	TGGGATGAAAAAGCGTTCTTTATCC ATGA	577	PGN_NC002163-327746- 328270_95_123_R	TGATAAAAAGCACTAAGCGATGAAACAG C	1178
2601	PGM_NC002163- 327746- 328270 220 250 F	TAAACACGGCTTTCCTATGGCTTATC CAAAT	146	PGM_NC002163-327746- 328270_314_345_R	TCAAGTSCTTTACTTCTATAGGTTTAA GCTC	963
2602	UNCA_NC002163- 112166- 112647 123 152 F	TOTAGCTTATCGCGAAATGTCTTTGA TTTT	628	UNCA_NC002163-112166- 112647_199_229_R	TGCTTGCTCTTTCAAGCAGTCTTGAATG AAG	1258
2603	UNCA_NC002163- 112166- 112647 333 365 F	TCCAGATOGACAAATTTTCTTAGAAA	313	UNCA MC002163-112166- 112647 430 461 R	TCCGARACTIGITITGTAGCTTTAATTT GAGC	1031
2734	GYRA_AY291534_237_2 64 F	TCACCCTCATGGTGATTCAGCTGTTT	265	GYRA AY291534 268 288 R	TIGGGCCATACGTACCATCGT	1407
2735	GYRA_AY291534_224_2 52 F	TAATCGGTAAGTATCACCCTCATGGT	167	GYRA AY291534 256 285 R	TGCCATACGTACCATCGTTCATAACA GC	1213
2736	GYRA_AY291534_170_1 98 F	TAGGAATTACGGCTGATAAAGCGTAT	221	GYRA AY291534 268 288 R	TIGCGCCATACGTACCATCGT	1407
2737	GYRA_AY291534_224_2 52 F	TAATCGGTAAGTATCACCCTCATGGT	167	GYRA AY291534 319 346 R	TATCGACAGATCCAAAGTTACCATGCCC	935
2738	GYRA NC002953-7005-	TAAGGTATGACACCGGATAAATCATA	163	GYRA_NC002953-7005- 9668_265_287_R	TCTTGAGCCATACGTACCATTGC	1142
2739	GYRA NC002953-7005-	TAATGGGTAAATATCACCCTCATGGT	171	GYRA NC002953-7005-	TATOCATTGAACCAAAGTTACCTTGGCC	933
2740	GYRA NC002953-7005-	TAATGGGTAAATATCACCCTCATGGT	171	GYRA_NC002953-7005- 9668_253_283_R	TAGCCATACGTACCATIGCTTCATAAAT AGA	912
2741	GYRA_NC002953-7005-	TCACCCTCATGGTGACTCATCTATT AT	264	GYRA_NC002953-7005- 9668_265_287_R	TCTTGAGCCATACGTACCATTGC	1142
2842	CAPC AF188935~ 56074- 55628 271 304 F	TGGGATTATTGITATCCTGTTATGCC ATTTGAGA	578	CAPC_AF188935-56074- 55628 348 378 R	IGGIAACCCTIGICTITGAATIGIATIT GCA	1299
2843	CAPC_AF188935- 56074- 55628 273 303P F	TGATTATTGTTATCCTGTTATGCpCp ATPTPTPGAG	476	CAPC_AF188935-56074- 55628 349 377P_R	TGTAACCCTTGTCTTTGAATpTpGTATp TpTpGC	1314
2864	CAPC_AF188935~ 56074~ 55628 268 303 F	TCCGTTGATTATTCTTATCCTGTTAT	331	CADC_AF188935-56074- 55628 349 384 R	TGTTAATGGTAACCCTTGTCTTTGAATT GTATTTGC	1344
2845	CAPC AF188935-	TOCGFIGATTATTGTTATCCTGTTAT	331	CAPC AF188935-56074-	TAACCCTTGTCTTTGAATTGTATTTGCA	860

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	852	889	1169	1001	1099	1162	1242	1069	1168	983	1144	1144	1237	965	1097	1210	1040	923	1060	1901
ATTAATCCTGG	TARAGGATAGCGGTAACTAAATGGCTGA GCCAT	TACCCCAGTICCCCTGACCTIC	TGAGCCATGAGTACCATGGCTTCATAAC	TCCRAGITIGACITRAACGIACCAICGC	TCGTCAACACTACCATACCATGCA TCTC	TGACTTAAACGTACCATCGCTTCATATA CAGA	TGCTAAAGTCTTGAGCCATACGAACAAT GG	TOGATOGRACOGRAGITACCCTGRCC	TORGCCATACGAACAATGGTTTCATAAA CAGC	TCAGCTGTTAACGGCTTCAAGACCC	TUTTIANGTICTICCAAGGAINGAITTA TITCIIGITCG	TUTUTAGETTCTTCCAAGGATAGATTTA TITCTTGTTCG	TGCGGTCTGGCGCATATAGGTA	TCAATCTCGACTTTTTGTGCCGGTA	TOGGITICAGICALCICCACCATAAAGG T	TGCCAGCGACAGCCATCGTA	TCCGGTAACTGGGTCAGCTCGAA	TRGTATCACCACGTACACCCGGGATCAGT	TCCTTTATGCAACTTGGTATCAACAGGA AT	TCCTTTATGCAACTTGGTATCAACCGGA AT
55628_337_375_R	PARC X95819 121 153 R	PARC X95819 157 178 R	PARC X95819 97 128 R	PARC_NC003997-3362578- 3365001_256_283_R	PARC_NC003997-3362578- 3365001_304_335_R	PARC NC003997-3362578- 3365001 244 275 R	GYRA AY642140 71 100 R	GYRA AY642140 121 146_R	GYRA AY642140 58 89 R	CYA AF065404 1448 1472 R	LEF BA AF065404 843 881 R	LEF BA AF065404 843 881 R	MUTS AY698802 172 193 R	NUTS AY698802 228 252 R	NUTS AY698802 314 342 R	NUTS AY698802 413 433 R	NUTS AY698802 497 519 R	AB MLST-11- OFF007 1110 1137 R	GAPA NC 002505 29 58 R 1	GAPA MC002505 769 798 2 R
	302	199	596	899	621	621	150	166	166	305	354	498	326	187	186	419	585	583	259	361
GCCATTIGAG	TCCharanatcaececeracaeree	TACTIGGTAAATACCACCCACATGGT	TGGTRAATACCACCCACATGGTGAC	TTCCGTAAGTCGGCTAAAACAGTCG	TGTPACTATCACCGCACGGTGAT	TGTPACTATCACCGCACGGTGAT	TAAATCTGCCCGTGTCGTTGGTGAC	TANTOGGTAAATATCACOCGCATGGT GAC	TRATCGGTRAATATCACCCGCATGGT	TCCRACGRAGTACAATACAAGACAAA	TOGRANGCITITGCAIRITATAIOGA	TGCATATTATATCGAGCCACAGCATC	TOCGCTGAATCTGTCGCCGC	TACCTATATGGGCCAGACGGC	TACCGGCGCAAAAAGTCCAGATTGG	TCTTTATGGTGGAGATGACTGAAACC	TGGGCGTGGAACGTCCAC	TGGGGGATGCTGCGAAATGGTTAAAA	TCAATGAACGACCAACAAGTGATTGA	TCGATGAACGACCAACAAGTGATTGA TG
56074-	PARC X95819 33 58 F		8	PARC NC003997- 3362578- 3365001 181 205 F	PARC_NC003997- 3362578- 3365001 217 240 F		GYRA AY642140	GYRA_AY642140_26_54	GYRA_AY642140_26_54	379 P	LEF BA AF065404_751	12F BA AP065404 762	MUTS_AY698802_106_1	NUTS_AY698802_172_1 92_F	MUTS_AY698802_228_2	MUTS_AY698802_315_3	NUTS_AX698802_394_4	AB MLST-11-	GAPA NC002505_694_7	GAPA NC002505 694_7
_	2846	2847	2848	2849	2850	2851	2852	2853	2854	2860	1386	2862	2917	2918	2919	2920	2921	500	2000	2928

DOCKET NO.: DIBIS-0083US1 (COUNSEL DOCKET NO: 10593)

1059	1410	1410	1410	3961	2	1341	1056	1001	1256	1259	1229	1111	978	176	1095	1376	
TCCTTTATGCAACTTAGTATCAACCGGA AT	TEGCEGCETTCGCAEGGTEAATCGCFFC AA	TRICTICTITICGCATGGTTAATCGCTTC	TINCITACITICGCATGGTTAATCGCTTC	000 640 640 000 400 400 400	- Taranta Caracter Ca	TGIGTTGTCGCCGCGCAG	TCCTTGGCATACATCATGTCGTAGCA	TCGGCGAACATGGCCATCAC	TGCTTCTCTCGGGTCGGC	TGCTTGGTGGCTTCTTCGTCGAA	TGGGAGGAACTTCACGTCCTGC	TCGTGGGCTTGCCGGT	TCACGGGCCAGCTCGTCT	TCACCATGCGCCCGTTCACATR	TCGGTGGTAGCCGATCTC	TICAGGTACAGCAGGTOGTTCAGGAT	
GAPA NC002505 769 798 3 R	INPB BC 1439 1468 R	INPB RC 1439 1468 R	INFB BC 1439 1468 R	ACS_NC002516-970624-	ARO NC002516-26883-	27380 111 128 R	27380 459 484 R	GUA_NC002516-4226546- 4226174 127 146 R	GUA NC002516-4226546- 4226174 214 233 R	GUA_NC002516-4226546- 4226174_265_287_R	GUN NC002516-4226546- 4226174 288 309 R	GUN NC002516-4226546- 4226174 355 371 R	MUT_NC002516-5551158- 5550717 99 116 R	MUT_NC002516-5551158- 5550717_256_277_R	NUO_NC002516-2984589- 2984954 97 117 R	NUO NC002516-2984589- 2984954 301 326 R	PPS NC002516-1915014-
361	889	689	685)	979	267	705	551	448	710	670	374	545	358	249	1.95	
GAFB_NC002505_694_7 TCGATGAACGACCAACAGTGATTGA 21.2 F	TIGCTOGIGGIGCACAGTAACGGAT	TIGCTCGTGGTGCALAGTAACGGAT	TTGCCCGCGCTGCGGAAGTAACCGAT		TORGOTOTOTOTO	TCACCGTGCCGTTCAAGGAAGAG	TTTCGAAGGGCCTTTCGACCTG	Tegacrecredgrege	TGACCAGGTGATGGCCATGTTCG	TTTTGAAGGTGATCCGTGCCAACG	TTCCTCBGCCBCCTBGC	TOGGCOGCACCTTCATCGAAGT	TGGAAGTCATCAAGCGCCTGGC	TCGAGGCGCTGCCG	TOPPOCHOENCO	TACTCTCGGTGGAGAAGCTCGC	
GAFA_NC002505_694_7	TNFB EC 1364 1394 P	INFB_EC_1364_1394_2 F	INFB EC 80 110 F	ACS NC002516- 970624-	ARO MC002516-26883-	27380 4 26 F	27380 356 377 F	GUA_NC002516- 4226546- 4226174 23 41 F	GUA_MC002516- 4226546- 4226174 120 142 F	GUA_MC002516- 4226546- 4226174 155 178 F	GUN_NC002516- 4226546- 4226174 190 206 F	GUN_NC002516- 4226546- 4226174 242 263 F	MUT_NC002516- 5551158- 5550717 5 26 F	MUT_NC002516- 5551158- 5550717 152 168 F	NUO NC002516- 2984589- 2984954 8 26 F	NUO NC002516- 2984589- 2984954 218 239 F	PPS_NC002516-
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1052	1011	1182	924	1094	1442	1008	1085	1085	1087	1086	1430	1026	1038	1225	982
TCCTGGCCATCCTGCAGGAT	TCGATCTCCTTGGCGTCCGA	TGATCTCCATGGCGCGGATCTT	TAGTATCACCACGTACICCIGGATCAGT	TCGGTCAGCBAAACGGTAGCTTGC	TTTTCCCTTTAIGCAACTTAGTAICAAC IGGAAT	TCCRIRCCTITALGCRACTIGIATCAA CIGGRAT	TOGGAAATATTCTTTCAATACCTTTATG CAACT	TOGGABATATTCTTTCAATACCTTTATG CAACT	TOGGRANIATICITICAATICCTITITG	TCGGAAATATTCTTTCAATACCTTTATG CAACTT	TITCAATACCITTAIGCAACTIIGIAIC AACIGGAAT	TCCCGGCTAGAGATTCTGTATACGA	TCCGGCTAGAGATTCTGTATACGAAAAT ATC	TGCCGIATACGRARATATCTTATCATTT AGCGT	TCHGCGTAGTCTAATAATTTACGGAACA TTTC
PPS_NC002516-1915014- 1915383 341_360_R	TRP_MC002516-671831- 672273_131_150_R	TRP_NC002516-671831- 672273 362 383 R	AB MLST-11- OIF007 1126 1153 R	OMPU NC002505 544 567 R	GAPA NC002505-506780- 507937_769_802_R	GAPA_NC002505-506780- 507937_769_803_R	GAPA NC002505-506780- 507937 785 817 R	GAPA NC002505-506780- 507937 785 817 R	GAPA NC002505-506780- 507937 784 817 R	GAPA_NC002505-506780- 507937 784 817 2 R	GAPA NC002505-506780- 507937 769 805 R	CTXB NC002505-1566967- 1567341 139 163 R	CTXB_MC002505-1566967- 1567341_132_162_R	CIXB_NC002505-1566967- 1567341_118_150_R	TUPB NC002758-615038- 616222 778 809 R
365	527	490	592	199	335	339	396	337	336	340	338	275	274	274	180
TCGCCATCGTCACCAACCG	TGCTGGTACGGGTCGAGGA	TGCACATOGTGTCCAC	TGGGIGATGCTGCIAAATGGTTAAAA GA	TTCCCACCGATATCATGGCTTACCAC	TCCTCAATGAACGAICAACAAGTGAT TGATG	TCCTCIATGAACGAICAACAAGTGAT TGATG	TCTCGATGAACGACCAACAAGTGATT GATG	TCCTCGATGAACGAICAACAAGTIAT TGATG	TCCTCAATGAATGATCAACAAGTGAT TGATG	TCCTCIATGAAIGAICAACAAGTIAT TGATG	TCCTCGATGAATGAICAACAAGTIAT TGATG	VOLOCULUCOSOLVO COLOCULOS VOLOS COLOCULOS VOLOS	TCAGCATATGCACATGGAACACCTC	TCAGCATATGCACATGGAACACCTC	TACAGGCCGTGTTGAACGTGG
1915014- 1915383 240 258 F	TRP_NC002516- 671831- 67273_24_42_F	TRP_NC002516- 671831- 67273 261 282 F	AB MLST-11- OIF007 1007 1034 F	OMPU_NC002505- 674828- 675880 428 455 F	GAPA_NC002505- 506780- 507937 691 721 F	GARA_NC002505- 506780- 507937 691 721 2 F	GAPA_NC002505- 506780- 507937 692 721 F	GAPA_NC002505- 506780- 507937 691 721 3 F	GAPA_NC002505- 506780- 507937 691 721 4 F	GAPA_NC002505- 506780- 507937 691 721 5 F	GAPA_NC002505- 506780- 507937 691 721 6 F	CTXB_NC002505- 1566967- 1567341 46 71 F	CTXB_NC002505- 1566967- 1567341 46 70 F	CTXB_NC002505- 1565967- 1567341 46 70 F	TUFB_MC002758- 615038-
2962	2963	2964	2972	2993	2994	2995	2996	2997	2998	2999	3000	3001	3002	3003	3004

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P TOCOGRATOLACTORATOLACTOR S10 TUTPS MODOTS6-615036-		616222 684 704 F					
Control Cont		TUPB_NC002758- 615038-			TUFB_NC002758-615038-	TGCTTCAGCGTAGTCTAATAATTTACGG	
Control Cont	2	616222 688 710 F	TGCCGTGTTGAACGTCGAAT	503	616222 783 813 R	AAC	1255
UNITED EXCORPTION CONTRIBUTION	y	TUFB_NC002758- 615038-	TGTGGTCAAATCAAAGTTGGTGAAGA	823	TUFB_NC002758-615038-	TGCGTAGTCTAATAATTTACGGAACATT	1238
6:6223 77.5 700TOBANTORANGEMANA 607 TUTP, \$605758 - 515.058 -		THE NOODSTEE					
TUTP NOD1755- ADARA	5		TGGTCAAATCAAAGTTGGTGAAGAA	607	TUPB_NC002758-615038- 616222_778_807_R	TGCGTAGTCTAATAATTTACGGAACATT TC	1238
THE RESIDENCE THE RESIDENC		TUFB_NC002758-	OTS DITTOR AS DATE OF THE OTHER PROPERTY OF		THER NC002758-615038-	TIGHTERTOTESTED PROTIDES CONT.	
TUTPS NOTOTITED ACCOUNTS NOTOT	80	396 726	AAGAA	431	616222 785 818 R	TACGGA	970
MIGGER MOROSARIA P. T. 1985 MIGGER MOROSARIA MIGGER MOROSARIA MIGGER MOROSARIA MIGGER		TUFB_NC002758-	TOPTGTTGBACGTGBAATCAAAG		TUPB NC002758-615038-	TCTTChactrattchactrattcaga	
MICH. R. NOROSSES SESTED CONCINTROPTOMOGRAMMACKER SESTED MICH. R. NOROSSES MICH. MICH. R. NOROSSES MICH. MIC	60	616222 690 716 ₹	E	386	616222 778 812 R	ACATITIC	1134
MIGT. MIGGO 3521-1778-1778-1778-1778-1778-1778-1778-17	9	MECI-R NC003923-	_	261	MECI-R NC003923-41798- 41609 89 112 R	TGTGATATGGAGGTGTAGAAGGTG	1332
Color Colo	١.	MECI-R NC003923-	-	100	MECI-R NC003923-41798-	TGGGATGTAGAGGTGTTATCA	1287
41759 20 7 7000.00.VNATOTOROROMACT 545 HEGT-R, 1000.9324-17796 41669 31 0.0 7 7000.00.VNATOTOROROMACT 545 HEGT-R, 1000.9324-17796 41669 31 0.0 7 7000.00.VNATOTOROROMACT 545 HEGT-R, 1000.9324-17796 41669 31 0.0 7 7000.00.VNATOTOROMACT 595 HEGT-R, 1000.9324-17796 4179 4-1600 31 7 7 7 7 7 7 7 7 7			+	500	2 024 10 0001		
Color Colo		41798-	TGGACACATATCGTGAGCAATGAACT		MECI-R_NC003923-41798-	TOGGATGGAGGTGTAGAGGTGTTATCA	1306
MICH MODISTAL MACHINICATIVE MACHINICAT	2	41609 33 60 2 F	GA	549	STRUE OT TIO K	,	200
MF 175	5	MECI-R_NC003923- 41798-41609 29 60 F		595	MECI-R NC003923-41798- 41609 81 113 R	TOGGGATATGGAGGTGTTA	1290
11 Pr. 77543-2450-25 700000000000000000000000000000000000		MUPR_X75439_2490_25	_	587	MITER X75419 2548 2570 R	TCTGGCTGCGGAAGTGGAAATCGT	1130
11 2 2 2 2 2 2 2 2 2		MITDD X75439 2490 25	100000000000000000000000000000000000000	3			
MURE_X75439_2482_55 TRAPATATGOGCTECTTCTCCCCTTA 205 MURE X75439_2552_2559_R MURE_X75439_2480_25 AAC MACRATGOGCTECTTCTCCCCTTAAACCCT S97 MURE X75439_2569_2579_R MURE_X75439_2480_25 TRAPATATGOGCTECTTCTCCCCTTAAACCCT AND X75439_2559_F MURE_X75439_2589_F MURE_X75439_2480_25 TRAPATATGOGCTECTTCTCCCCTTAAACCCT AND X75439_2559_259_F MURE_X75439_2480_25 TRAPATATGOGCTECTTCTCCCCTTAAACCCT AND X75439_2559_259_F MURE_X75439_2480_25 TRAPATATGOGCTECTTCTCCCCTTAAACCCT AND X75439_2559_259_F MURE_X75439_2480_25 TRAPATATGOGCTECTTCTCCCTTAAACCCT AND X75439_2559_159_F MURE_X75439_2480_25 TRAPATATGOGCTECTTCTCCCTTAAACCCT AND X75439_2559_159_F MURE_X75439_2480_25 TRAPATATGOGCTECTTCTCCCTTAAACCCT AND X75439_2559_F MURE_X75439_2480_25 TRAPATATGOGCTECTTCTCCCTTAAACCCT AND X75439_2559_F MURE_X75439_2480_25 TRAPATATGOGCTECTTCTCCCTTAAACCCT AND X75439_2559_F MURE_X75439_2480_25 TRAPATATGOGCTECTTCTCCCTTAAACCCT AND X75439_2559_F MURE_X75439_2480_25 TRAPATATGOGCTECTTCTCCCTTAAACCCT AND X75439_2559_2559_F MURE_X75439_259_259_F TRAPATATGOGCTECTTCTCCCTTAAACCCT AND X75439_259_259_F MURE_X75439_259_259_F TRAPATATGOGCTECTTCTCCCTTAAACCCT AND X75439_259_259_F MURE_X75439_259_259_F TRAPATATGOGCTECTTCTCCCTTAAACCCT AND X75439_259_259_F MURE_X75439_F AND X75439_259_259_F AND X75439_259_259_F MURE_X75439_F AND X75439_F	13	13 F	TGGGCTCTTTCTCGCTTAAACACC	286	NUPR X75439 2547 2568 R	TGGCTGCGGAAGTGAAATCGTA	1281
MURY X7543 2490_35 YGGGCCUTTVCTCGCTTAAACACCT SB77 MURY X75439 2549 2573 R 10	16	MUPR_X75439_2482_25	-	205	NUPR X75439 2551 2573 R	TAATCTGGCTGCGGAAGTGAAAT	876
MURP_XT613_2462_25 AACT AAATTGGGGTCTTTTCTCGTTA 2005 MURP XT5139 2559_2559_1559_R 10	12	MUPR_X75439_2490_25	TGGGCTCTTTCTCGCTTAAACACCT	587	MUPR X75439 2549 2573 R	TARTCTGGCTGCGGAAGTGAMTCG	877
MARK 751613_2150_255 TOGGETUTTECTOGETURALOCET 587 MURR X75435 2554_251 R MORR X75435 2554_251 R MORR X75435 2554_2551 R MORR X75435_2554_2551 R MORR X75435_2554_2554_2554_2554_2554_2554_2554_	α.	10 P		205	MUPR X75439 2559 2589 R	TOGININITCGITANITANICIGGCIGC GGA	1303
MONE NEOD322	9	MUPR_X75439_2490_25	TOGGCTCTTTCTCGCTTPAACACCT	587	MUPR X75439 2554 2581 R	TCGTTAATTAATCTGGCTGCGGAAGTGA	1112
NACO 1770 252			TGATGGCAAGTGGATAGGGTATAATA	į,	AROE MC003923-1674726-	TOGGOSTATION	868
16/47726 16/47726 16/47727 16/47726 16/47727 16/47726 16/47727 16/47727 16/47727 16/47727 16/47727 16/4777 16/47727 16/477 16/477	3		200	111	200 000 000		
AND MODISOR - REGIONAL MODERNING - 1674726-	2	1674726-	пессаветнаавтранадрадуюма	570	AROE NC003923-1674726-	ITCATAAGCAATACCTITACTIGCACCA C	1378
G Gloco are checked and an analysis of the checked and an anal		AROE NC003923-	TGGCpAAGTpGGATpAGGGTpATpAA	cha	AROE NC003923-1674726-	TARGCATACCPTPTPTPACTPTPGCPA	298
		200					

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	1674277_207_232P_F					
	ARCC NC003923-					
	2725050-	TCTGARATGAATAGTGATAGAACTGT		ARCC NC003923-2725050-	TCTTCTTCGTATAAAAGGACCAA	
3023	2724595 124 155 F	AGGCAC	398	2724595 214 245 R	TIGG	1137
	ARCC NC003923-					
	2725050-	TGRATAGTGATAGAACTGTAGGCACA		ARCC NC003923-2725050-	TCTTCTTTCGTATAAAAGGACCAATTG	
3024	2724595 131 161 F	ATOGI	437	2724595 212 242 R	CIL	1139
	ARCC NC003923-					
	2725050-	TGAATAGIGATAGAACTGTAGGCACA		ARCC NC003923-2725050-	IGCGCTAATTCTICAACTICTICTITCG	
3025	2724595 131 161 F	ATOGT	437	2724595 232 260 R	H	1232
	PTA NC003923-					
	628885-	TACAATGCTTGTTTATGCTGGTAAAG		PTA NC003923-628885-	TGTTCTTGATACACCTGGTTTCGTTTTTG	
3026	629355 231 259 F	CAG	177	629355 322 351 R	AT	1350
	PTA NC003923-					
	628885-	TACAATGCTTGTTTATGCTGGTAAAG		PTA NC003923-628885-	TGGTACACCTGGTTTCGTTTTGATGATT	
3027	629355 231 259 F	CAG	177	629355 314 345 R	TGTA	1301
	PIR NC003923-					
	628885-	TCTTGTTTATGCTGGTAAAGCAGATG		PTA NC003923-628885-	TGTTCTTGATACACCTGGTTTCGTTTTG	
0000		_			-	

[370] Primer pair name codes and reference sequences are shown in Table 3. The primer name code typically represents the gene to which the given primer pair is targeted. The primer pair name may include specific coordinates with respect to a reference sequence defined by an extraction of a section of sequence or defined by a GenBank gi number, or the corresponding complementary sequence of the extraction, or the entire GenBank gi number as indicated by the label "no extraction." Where "no extraction" is indicated for a reference sequence, the coordinates of a primer pair named to the reference sequence are with respect to the GenBank gi listing. Gene abbreviations are shown in bold type in the "Gene Name" column.

To determine the exact primer hybridization coordinates of a given pair of primers on a given bioagent nucleic acid sequence and to determine the sequences, molecular masses and base compositions of an amplification product to be obtained upon amplification of nucleic acid of a known bioagent with known sequence information in the region of interest with a given pair of primers, one with ordinary skill in bioinformatics is capable of obtaining alignments of the primers of the present invention with the GenBank gi number of the relevant nucleic acid sequence of the known bioagent. For example, the reference sequence GenBank gi numbers (Table 3) provide the identities of the sequences which can be obtained from GenBank. Alignments can be done using a bioinformatics tool such as BLASTn provided to the public by NCBI (Bethesda, MD). Alternatively, a relevant GenBank sequence may be downloaded and imported into custom programmed or commercially available bioinformatics programs wherein the alignment can be carried out to determine the primer hybridization coordinates and the sequences, molecular masses and base compositions of the amplification product. For example, to obtain the hybridization coordinates of primer pair number 2095 (SEO ID NOs; 456:1261), First the forward primer (SEO ID NO: 456) is subjected to a BLASTn search on the publicly available NCBI BLAST website. "RefSeq Genomic" is chosen as the BLAST database since the gi numbers refer to genomic sequences. The BLAST query is then performed. Among the top results returned is a match to GenBank gi number 21281729 (Accession Number NC 003923). The result shown below, indicates that the forward primer hybridizes to positions 1530282..1530307 of the genomic sequence of Staphylococcus aureus subsp. aureus MW2 (represented by gi number 21281729).

```
Staphylococcus aureus subsp. aureus MW2, complete genome
Length=2820462
Features in this part of subject sequence:
Panton-Valentine leukocidin chain F precursor
```

Score = 52.0 bits (26), Expect = 2e-05 Identities = 26/26 (100%), Gaps = 0/26 (0%)

[372] The hybridization coordinates of the reverse primer (SEQ ID NO: 1261) can be determined in a similar manner and thus, the bioagent identifying amplicon can be defined in terms of genomic coordinates. The query/subject arrangement of the result would be presented in Strand = Plus/Minus format because the reverse strand hybridizes to the reverse complement of the genomic sequence. HThe preceding sequence analyses are well known to one with ordinary skill in bioinformatics and thus, Table 3 contains sufficient information to determine the primer hybridization coordinates of any of the primers of Table 2 to the applicable reference sequences described therein.

Table 3: Primer Name Codes and Reference Sequence

			Reference
			GenBank gi
Primer name code	Gene Name	Organism	number
16S_EC	16S TRNA (16S ribosomal RNA gene)	Escherichia coli	16127994
23S_EC	23S rRNA (23S ribosomal RNA gene)	Escherichia coli	16127994
CAPC_BA	capC (capsule biosynthesis gene)	Bacillus anthracis	6470151
CYA_BA	cya (cyclic AMP gene)	Bacillus anthracis	4894216
DNAK_EC	dnaK (chaperone dnaK gene)	Escherichia coli	16127994
GROL_EC	groL (chaperonin groL)	Escherichia coli	16127994
	hflb (cell division protein peptidase	Escherichia coli	
HFLB_EC	ftsH)		16127994
	infB (protein chain initiation factor	Escherichia coli	
INFB_EC	infB gene)		16127994
LEF_BA	lef (lethal factor)	Bacillus anthracis	21392688
PAG_BA	pag (protective antigen)	Bacillus anthracis	21392688
RPLB_EC	rplB (50S ribosomal protein L2)	Escherichia coli	16127994
	rpoB (DNA-directed RNA polymerase beta	Escherichia coli	
RPOB_EC	chain)		6127994
	rpoC (DNA-directed RNA polymerase	Escherichia coli	
RPOC_EC	beta' chain)		16127994
	Artificial Sequence Concatenation comprising:	Artificial Sequence* -	
	gki (glucose kinase)	partial gene sequences of Streptococcus	
SP101ET_SPET_11	gtr (glutamine transporter protein)	pyogenes	15674250
	murI (glutamate racemase)		
	muts (DNA mismatch repair protein)		
	xpt (xanthine phosphoribosyl transferase)		
	yqiL (acetyl-CoA-acetyl transferase)		
	tkt (transketolase)		
	sspE (small acid-soluble spore		
SSPE_BA	protein)	Bacillus anthracis	30253828
TUFB_EC	tufB (Elongation factor Tu)	Escherichia coli	16127994

VALS_EC	valS (Valy1-tRNA synthetase)	Escherichia coli	16127994
ASPS EC	aspS (Asparty1-tRNA synthetase)	Escherichia coli	16127994
CAF1 AF053947	cafl (capsular protein cafl)	Yersinia pestis	2996286
INV U22457	inv (invasin)	Yersinia pestis	1256565
INV_U22457	Y. pestis specific chromosomal genes -	rersinia pestis	16120353
LL NC003143	difference region	Yersinia pestis	
Donney Manage	BoNT/A (neurotoxin type A)	Clostridium botulinum	40381
BONTA_X52066	BONT/A (neurocoxin type A)	Stanhylococcus	2791983
MECA_Y14051	mecA methicillin resistance gene	aureus	
	trpE (anthranilate synthase (large	Acinetobacter	20853695
TRPE_AY094355	component))	baumanii	
RECA AF251469	recA (recombinase A)	Acinetobacter baumanii	9965210
RECK MEZDI469	Tech (Tecomornase A)	Acinetobacter	4240540
GYRA_AF100557	gyrA (DNA gyrase subunit A)	baumanii	
GYRB AB008700	gyrB (DNA gyrase subunit B)	Acinetobacter baumanii	4514436
GIRD_AGOUG 700	waah (3-deoxy-D-manno-octulosonic-acid	Acinetobacter	2765828
WAAA_Z96925	transferase)	baumanii	2705020
	Artificial Sequence Concatenation		
	comprising:		
	tkt (transketolase)		15791399
	and (examinational)		
	glyA (serine hydroxymethyltransferase)		
CUST_CU		Artificial	
	gltA (citrate synthase)	Sequence* -	
	aspA (aspartate ammonia lyase)	partial gene sequences of	
	aspa (asparcace ammonia lyase)	Campylobacter	
	glnA (glutamine synthase)	jejuni	
	pgm (phosphoglycerate mutase)		
	uncA (ATP synthetase alpha chain)		
		Bordetella	33591275
RNASEP_BDP	RNase P (ribonuclease P)	pertussis	
		Burkholderia	53723370
RNASEP_BKM	RNase P (ribonuclease P) RNase P (ribonuclease P)	mallei	
RNASEP_BS	RNase P (ribonuclease P)	Bacillus subtilis Clostridium	16077068
RNASEP CLB	RNase P (ribonuclease P)	perfringens	18308983
RNASEP EC	RNase P (ribonuclease P)	Escherichia coli	16127994
RNASEP EC	Awase F (Liboliuclease F)	Rickettsia	15603881
RNASEP RKP	RNase P (ribonuclease P)	prowasekii	13003661
	Talada I (Ilabalinazania I)	Staphylococcus	15922990
RNASEP SA	RNase P (ribonuclease P)	aureus	
RNASEP VBC	RNase P (ribonuclease P)	Vibrio cholerae	15640032
ICD_CXB	icd (isocitrate dehydrogenase)	Coxiella burnetii	29732244
		Acinetobacter	29732244
IS1111A	multi-locus IS1111A insertion element	baumannii	
OWDS SWARESS	ompA (outer membrane protein A)	Rickettsia prowazekii	40287451
OMPA_AY485227	ompa (outer memorane protein A)	prowazekii Rickettsia	15603881
OMPB RKP	ompB (outer membrane protein B)	prowazekii	12002881
GLTA RKP	gltA (citrate synthase)	Vibrio cholerae	15603881
		Francisella	15640032
TOXR VBC			
	toxR (transcription regulator toxR)	tularensis	
	asd (Aspartate semialdehyde	Francisella	56707187
ASD FRT	asd (Aspartate semialdehyde dehydrogenase)	Francisella tularensis	
	asd (Aspartate semialdehyde	Francisella tularensis Shigella flexneri	56707187
ASD_FRT GALE_FRT	asd (Aspartate semialdehyde dehydrogenase) galE (UDP-glucose 4-epimerase)	Francisella tularensis Shigella flexneri Campylobacter	
ASD FRT	asd (Aspartate semialdehyde dehydrogenase)	Francisella tularensis Shigella flexneri Campylobacter jejuni	56707187
ASD FRT GALE_FRT IPAH SGF	asd (Aspartate semialdehyde dehydrogenase) gal8 (UDP-glucose 4-epimerase) ipaH (invasion plasmid antigen)	Francisella tularensis Shigella flexneri Campylobacter	56707187 30061571
ASD FRT GALE_FRT IPAH SGF HUPB_CJ	asd (Aspartate semialdehyde dehydrogenase) gal8 (UDF-glucose 4-epimerase) ipaH (invasion plasmid antigen) hup8 (DNA-binding protein Hu-beta)	Francisella tularensis Shigella flexneri Campylobacter jejuni	56707187 30061571 15791399
ASD FRT GALE_FRT IPAH SGF	asd (Aspartate semialdehyde dehydrogenase) gal8 (UDP-glucose 4-epimerase) ipaH (invasion plasmid antigen)	Francisella tularensis Shigella Flexneri Campylobacter jejuni Coxiella burnetii	56707187 30061571 15791399 Sequenced in-house
ASD FRT GALE_FRT IPAH SGF HUPB_CJ	asd (Aspartate semialdehyde dehydrogenase) 9alE (UDP-Glucose 4-epimerase) ipaH (invasion plasmid antigen) hupB (DNA-binding protein Hu-beta) Artificial Sequence Conactenation	Francisella tularensis Shigella flexneri Campylobacter jejuni Coxiella burnetii	56707187 30061571 15791399 Sequenced

1	I))	sequences of Acinetobacter	
Į.	adk (adenylate kinase)	baumannii	
	auk (adenylace kinase)	Damminit	
	mutY (adenine glycosylase)		
	J.,,		
	fumC (fumarate hydratase)		
	efp (elongation factor p)		
	ppa (pyrophosphate phospho-		
	hydratase		
		Staphylococcus	
MUPR X75439	mupR (mupriocin resistance gene)	stapnylococcus aureus	438226
MUPR_X/5439	maps (mapriocin resistance gene)	Acinetobacter	430220
PARC X95819	parC (topoisomerase IV)	baumannii	1212748
Time Appear	para (depositional str)	Staphylococcus	
SED M28521	sed (enterotoxin D)	aureus	1492109
PLA AF053945	pla (plasminogen activator)	Yersinia pestis	2996216
		Staphylococcus	
SEJ_AF053140_	sej (enterotoxin J)	aureus	3372540
		Mycoplasma	
GYRA_NC000912	gyrA (DNA gyrase subunit A)	pneumoniae	13507739
		Pseudomonas	
ACS_NC002516_	acsA (Acetyl CoA Synthase)	aeruginosa	15595198
		Pseudomonas	
ARO_NC002516	aroE (shikimate 5-dehydrogenase	aeruginosa	15595198
	(com)	Pseudomonas aeruginosa	15595198
GUA_NC002516	gusA (GMP synthase)	Pseudomonas	12232130
MUT NC002516	muth (DNA mismatch repair protein)	aeruginosa	15595198
NOT NC002516	Much (DRA mismacch repair process)	Pseudomonas	20000000
NUO NC002516	muoD (NADH dehydrogenase I chain C, D)	aeruginosa	15595198
Neo_Neocopaco		Pseudomonas	
PPS_NC002516	ppsA (Phosphoenolpyruvate synthase)	aeruginosa	15595198
	trpE (Anthranilate synthetase	Pseudomonas	
TRP_NC002516	component I)	aeruginosa	15595198
		Chlamydia	
OMP2_NC000117	ompB (outer membrane protein B)	trachomatis	15604717
		Chlamydia	
OMPA_NC000117	ompA (outer membrane protein B)	trachomatis	15604717
		Chlamydia trachomatis	15604717
GYRA_NC000117	gyrA (DNA gyrase subunit A) ctxA (Cholera toxin A subunit)	Vibrio cholerae	15640032
CTXA_NC002505	ctxB (Cholera toxin B subunit)	Vibrio cholerae	15640032
FUR NC002505	fur (ferric uptake regulator protein)	Vibrio cholerae	15640032
FOR_NC002303	gapA (glyceraldehyde-3-phosphate	FIDITO CHOICING	13040035
GAPA NC 002505	dehydrogenase)	Vibrio cholerae	15640032
GYRB NC002505	gyrB (DNA gyrase subunit B)	Vibrio cholerae	15640032
OMPU NC002505	ompU (outer membrane protein)	Vibrio cholerae	15640032
TCPA NC002505	tcpA (toxin-coregulated pilus)	Vibrio cholerae	15640032
		Campylobacter	
ASPA_NC002163	aspA (aspartate ammonia lyase)	jejuni	15791399
		Campylobacter	
GLNA_NC002163	glnA (glutamine synthetase)	jejuni	15791399
		Campylobacter	
GLTA_NC002163	gltA (glutamate synthase)	jejuni	15791399
		Campylobacter	
GLYA_NC002163	glyA (serine hydroxymethyltransferase)	jejuni	15791399
		Campylobacter	
PGM_NC002163	pgm (phosphoglyceromutase)	jejuni Campylobacter	15791399
mym NGGGGGGG	tkt (transketolase)		15791399
TKT_NC002163	CKL (CIAMSKECOLASE)	jejumi Campylobacter	15/91399
UNCA NC002163	uncA (ATP synthetase alpha chain)	jejuni	15791399
ONCM_MCUUZI63	mes (ers, shirenecase arbug cuqui)	Staphylococcus	13/513/9
AGR-III NC003923	agr-III (accessory gene regulator-III)	aureus	21281729
111_10003923		Staphylogoggus	1
ARCC NC003923	arcC (carbamate kinase)	aureus	21281729
AROE NC003923	aroE (shikimate 5-dehydrogenase	Staphylococcus	21281729

		aureus	
		Staphylococcus	
BSA-A NC003923	bsa-a (glutathione peroxidase)	aureus	21281729
	bsa-b (epidermin biosynthesis protein	Staphylococcus	
BSA-B NC003923	EpiB)	aureus	21281729
		Staphylogoggus	
GLPF NC003923	glpF (glycerol transporter)	aureus	21281729
GHFF_MC003923	gips (gryceror cramaporcer)	Staphylococcus	STROTIES
	omk (quanvlate kinase)		*****
GMK_NC003923		aureus	21281729
	mecR1 (truncated methicillin	Staphylococcus	
MECI-R_NC003923	resistance protein)	aureus	21281729
		Staphylococcus	
PTA NC003923	pta (phosphate acetyltransferase)	aureus	21281729
	pvluk (Panton-Valentine leukocidin	Staphylococcus	
PVLUK NC003923	chain F precursor)	aureus	21281729
11000 10010100	ondan'i pandangany	Staphylococcus	040-07-0
SA442 NC003923	sa442 gene	aureus	21281729
SA442 NC003923			21201/23
1	sea (staphylococcal enterotoxin A	Staphylococcus	
SEA_NC003923	precursor)	aureus	21281729
		Staphylococcus	
SEC_NC003923	sec4 (enterotoxin type C precursor)	aureus	21281729
		Staphylococcus	
TPI NC003923	tpi (triosephosphate isomerase)	aureus	21281729
	vgi (acetyl-CoA C-acetyltransferase	Staphylococcus	
YQI NC003923	homoloque)	aureus	21281729
191 40003523	nonorogue)	Francisella	22202727
GALE AF513299	galE (galactose epimerase)	tularensis	23506418
		Vibrio vulnificus	
VVHA_NC004460	vVhA (cytotoxin, cytolysin precursor)		27366463
		Vibrio	
TDH_NC004605	tdh (thermostable direct hemolysin A)	parahaemolyticus	28899855
		Staphylococcus	
AGR-II NC002745	agr-II (accessory gene regulator-II)	aureus	29165615
PARC NC003997	parC (topoisomerase IV)	Bacillus anthracis	30260195
GYRA AY291534	gyrA (DNA gyrase subunit A)	Bacillus anthracis	31323274
GIRK_HILDIDG	gyan (bin gyanse submise n)	Staphylococcus	52525074
	agr-I (accessory gene regulator-I)		
AGR-I_AJ617706	agr-1 (accessory gene regulator-1)	aureus	46019543
		Staphylococcus	
AGR-IV_AJ617711	agr-IV (accessory gene regulator-III)	aureus	46019563
		Staphylococcus	
AGR-IV_AJ617711 BLAZ_NC002952	agr-IV (accessory gene regulator-III) blaZ (beta lactamase III)		46019563 49482253
		Staphylococcus	
BLAZ_NC002952	blaZ (beta lactamase III)	Staphylococcus aureus	
		Staphylococcus aureus Staphylococcus aureus	49482253
BLAZ_NC002952 ERMA_NC002952	blaZ (beta lactamase III) ermA (rRNA methyltransferase A)	Staphylococcus aureus Staphylococcus aureus Staphylococcus	49482253 49482253
BLAZ_NC002952	blaZ (beta lactamase III) ermA (rRNA methyltransferase A) ermB (rRNA methyltransferase B)	Staphylococcus aureus Staphylococcus aureus Staphylococcus aureus	49482253
BLAZ_NC002952 ERMA_NC002952 ERMB_Y13600	blaZ (beta lactamase III) ermA (rRNA methyltransferase A) ermB (rRNA methyltransferase B) sea (staphylococcal enterotoxin A	Staphylococcus aureus Staphylococcus aureus Staphylococcus aureus Staphylococcus	49482253 49482253 49482253
BLAZ_NC002952 ERMA_NC002952	blaz (beta lactamase III) ermà (rRNA methyltransferase A) ermB (rRNA methyltransferase B) sea (staphylcoccal enterotoxin A precursor)	Staphylococcus aureus Staphylococcus aureus Staphylococcus aureus Staphylococcus aureus aureus	49482253 49482253
BLAZ_NC002952 ERMA_NC002952 ERMB_Y13600 SEA-SEE_NC002952	blaz (beta lactamase III) ermA (rRNA methyltransferase A) ermB (rRNA methyltransferase B) sea (staphylococal enterotoxin A precursor) sea (staphylococal enterotoxin A	Staphylococcus aureus Staphylococcus aureus Staphylococcus aureus Staphylococcus aureus Staphylococcus Staphylococcus	49482253 49482253 49482253 49482253
BLAZ_NC002952 ERMA_NC002952 ERMB_Y13600	blaZ (beta lactamase III) ermA (rRNA methyltransferase A) ermB (rRNA methyltransferase B) sea (staphylococcal enterctoxin A precursor) sea (staphylococcal enterctoxin A precursor)	Staphylococcus aureus Staphylococcus aureus Staphylococcus aureus Staphylococcus aureus Staphylococcus aureus	49482253 49482253 49482253
BLAZ NC002952 ERMA NC002952 ERMB Y13600 SEA-SEE NC002952 SEA-SEE NC002952	blaz (beta lactamase III) ermA (rRNA methyltransferase A) erms (rRNA methyltransferase B) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A presumox)	Staphylococcus aureus Staphylococcus aureus Staphylococcus aureus Staphylococcus aureus Staphylococcus aureus Staphylococcus aureus Staphylococcus	49482253 49482253 49482253 49482253
BLAZ_NC002952 ERMA_NC002952 ERMB_Y13600 SEA-SEE_NC002952	blaZ (beta lactamase III) ermA (rRNA methyltransferase A) ermB (rRNA methyltransferase B) sea (staphylococcal enterctoxin A precursor) sea (staphylococcal enterctoxin A precursor)	Staphylococcus aureus	49482253 49482253 49482253 49482253
BLAZ_NC002952 ERMA_NC002952 ERMB_Y13600 SEA-SEE_NC002952 SEA-SEE_NC002952 SEE_NC002952	blaz (beta lactamase III) ermA (rRMA methyltransferase A) ermS (rRMA methyltransferase B) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor)	Staphylococcus aureus Staphylococcus Staphylococcus Staphylococcus Staphylococcus	49482253 49482253 49482253 49482253 49482253 49482253
BLAZ NC002952 ERMA NC002952 ERMB Y13600 SEA-SEE NC002952 SEA-SEE NC002952	blaz (beta lactamase III) ermA (rRNA methyltransferase A) erms (rRNA methyltransferase B) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A presumox)	Staphylococcus aureus	49482253 49482253 49482253 49482253
BLAZ_NC002952 ERMA_NC002952 ERMB_Y13600 SEA-SEE_NC002952 SEA-SEE_NC002952 SEE_NC002952	blaz (beta lactamase III) ermA (rRMA methyltransferase A) ermS (rRMA methyltransferase B) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor)	Staphylococcus aureus Staphylococcus Staphylococcus Staphylococcus Staphylococcus	49482253 49482253 49482253 49482253 49482253 49482253
BLAZ_NC002952 ERMA_NC002952 ERMB_Y13600 SEA-SEE_NC002952 SEA-SEE_NC002952 SEE_NC002952	blaz (beta lactamase III) ermA (rRMA methyltransferase A) ermS (rRMA methyltransferase B) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor)	Staphylococcus aureus Staphylococcus sureus Staphylococcus aureus Staphylococcus aureus Staphylococcus aureus	49482253 49482253 49482253 49482253 49482253 49482253
BLAZ NC002952 ERMA NC002952 ERMB Y13600 SEA-SEE NC002952 SEA-SEE NC002952 SEE NC002952 SEH NC002953 ERMC NC003908	blaz (beta lactamase III) ermA (rENA methyltransferase A) erms (rENA methyltransferase B) erms (rENA methyltransferase B) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor) eth (staphylococcal enterotoxin A precursor) eth (staphylococcal enterotoxin H) ermC (rENA methyltransferase C)	Staphylococus sureus	49482253 49482253 49482253 49482253 49482253 49482253 49484912 49489772
BLAZ NC002952 ERMA NC002952 ERMA Y13600 SEA-SEE NC002952 SEA-SEE NC002952 SEE NC002953	blaz (beta lactamase III) ermA (rRMA methyltransferase A) ermB (rRMA methyltransferase B) sea (staphylococcal enterctoxin A precursor) sea (staphylococcal enterctoxin A precursor) seh (staphylococcal enterctoxin A precursor)	Staphylococcus aureus Staphylococcus saureus	49482253 49482253 49482253 49482253 49482253 49482253 49482253
BLAZ NC002952 ERMA NC002952 ERMS Y13600 SEA-SEE NC002952 SEA NC002952 SEE NC002952 SEH NC002953 ERMC NC005908 MUTS AY69802	blaz (beta lactamase III) ermA (rBMA methyltransferase A) ermB (rBMA methyltransferase B) ermB (rBMA methyltransferase B) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor) erm (rBMA methyltransferase C) mutS (DNA mismatch repair protein)	Staphylococcus aureus Staphylococcus	49482253 49482253 49482253 49482253 49482253 49482253 49482253 49489772 52698233
BLAZ NC002952 ERMA NC002952 ERMB Y13600 SEA-SEE NC002952 SEA-SEE NC002952 SEE NC002952 SEH NC002953 ERMC NC003908	blaz (beta lactamase III) ermA (rENA methyltransferase A) erms (rENA methyltransferase B) erms (rENA methyltransferase B) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor) eth (staphylococcal enterotoxin A precursor) eth (staphylococcal enterotoxin H) ermC (rENA methyltransferase C)	Staphylococus aureus Staphylococus aureus Staphylococus saureus Staphylococus saureus Staphylococus saureus Staphylococus sureus Staphylococus sureus Staphylococus sureus Staphylococus Staphylococus Staphylococus Staphylococus Staphylococus Staphylococus Staphylococus Staphylococus sureus Staphylococus sureus snigella boydii	49482253 49482253 49482253 49482253 49482253 49482253 49484912 49489772
BLAE NC002952 ERMA NC002952 ERMB Y13600 SEA-SEE NC002952 SEA-SEE NC002952 SEE NC002952 SEH NC002953 ERMC NC005968 MUTS AY698502 NUC NC002758	blaz (beta lactamase III) ermA (rRNA methyltransferase A) erms (rRNA methyltransferase B) erms (rRNA methyltransferase B) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin B) ermc (rRNA methyltransferase C) mutS (DNA mismatch repair protein) nuc (staphylococcal nuclease)	Staphylococcus sureus Staphylococcus sureus Staphylococcus sureus sureus sureus sureus sureus sureus staphylococcus staphylococcus staphylococcus staphylococcus staphylococcus staphylococcus sureus staphylococcus sureus staphylococcus sureus staphylococcus sureus staphylococcus sureus staphylococcus sureus	49482253 49482253 49482253 49482253 49482253 49482253 49482253 49484912 49489772 52698233 57634611
BLAZ NC002952 ERMA NC002952 ERMS Y13600 SEA-SEE NC002952 SEA NC002952 SEE NC002952 SEH NC002953 ERMC NC005908 MUTS AY69802	blaz (beta lactamase III) ermA (rBMA methyltransferase A) ermB (rBMA methyltransferase B) ermB (rBMA methyltransferase B) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor) erm (rBMA methyltransferase C) mutS (DNA mismatch repair protein)	Staphylococcus saureus Staphylococcus	49482253 49482253 49482253 49482253 49482253 49482253 49482253 49489772 52698233
BLAE NC002952 ERMA NC002952 ERMB Y13600 SEA-SEE NC002952 SEE NC002952 SEE NC002952 SEE NC002952 SEE NC002953 ERMC NC005908 MUTS A1698802 NUC NC002758 SEE NC002758	blaz (beta lactamase III) ermA (rENA methyltransferase A) erms (rENN methyltransferase B) sea (staphylococcal enterotoxin A precursor) seh (staphylococcal enterotoxin B) ermC (rENA methyltransferase C) muts (DNA mismatch repair protein) muc (staphylococcal muclease) seb (enterotoxin type B precursor)	Staphylococcus aureus Staphylococcus auxeus Staphylococcus	49482253 49482253 49482253 49482253 49482253 49482253 49482253 49484912 49489772 52698233 57634611
BLAE NC002952 ERMA NC002952 ERMB Y13600 SEA-SEE NC002952 SEA-SEE NC002952 SEE NC002952 SEH NC002953 ERMC NC005968 MUTS AY698502 NUC NC002758	blaz (beta lactamase III) ermA (rRNA methyltransferase A) erms (rRNA methyltransferase B) erms (rRNA methyltransferase B) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin B) ermc (rRNA methyltransferase C) mutS (DNA mismatch repair protein) nuc (staphylococcal nuclease)	Staphylococcus surveus Staphylococcus surveus Staphylococcus	49482253 49482253 49482253 49482253 49482253 49482253 49482253 49484912 49489772 52698233 57634611
BLAE NC002952 ERMA NC002952 ERMB Y13600 SEA-SEE NC002952 SEE NC002952 SEE NC002952 SEE NC002952 SEE NC002953 ERMC NC005908 MUTS A1698802 NUC NC002758 SEE NC002758	blaz (beta lactamase III) ermA (rENA methyltransferase A) erms (rENN methyltransferase B) sea (staphylococcal enterotoxin A precursor) seh (staphylococcal enterotoxin B) ermC (rENA methyltransferase C) muts (DNA mismatch repair protein) muc (staphylococcal muclease) seb (enterotoxin type B precursor)	Staphylococcus aureus Staphylococcus auxeus Staphylococcus	49482253 49482253 49482253 49482253 49482253 49482253 49482253 49484912 49489772 52698233 57634611
BLAE NC002952 ERMA NC002952 ERMB Y13600 SEA-SEE NC002952 SEE NC002952 SEE NC002952 SEE NC002952 SEE NC002953 ERMC NC005908 MUTS A1698802 NUC NC002758 SEE NC002758	blaz (beta lactamase III) ermA (rENA methyltransferase A) erms (rENN methyltransferase B) sea (staphylococcal enterotoxin A precursor) seh (staphylococcal enterotoxin B) ermC (rENA methyltransferase C) muts (DNA mismatch repair protein) muc (staphylococcal muclease) seb (enterotoxin type B precursor)	Staphylococcus surveus Staphylococcus surveus Staphylococcus	49482253 49482253 49482253 49482253 49482253 49482253 49482253 49484912 49489772 52698233 57634611
BLAE_NC002952 ERMA_NC002952 ERMB_Y13600 SEA-SEE_NC002952 SEE_NC002952 SEE_NC002952 SEE_NC002952 SEE_NC002953 ERMC_NC005968 MUTS_A74598802 NUC_NC002758 SEB_NC002758 SEB_NC002758	blaz (beta lactamase III) ermA (rENA methyltransferase A) ermS (rENA methyltransferase B) sea (staphylococcal enterotoxin A resea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin B ermC (rENA methyltransferase C) muts (DNA miswatch repair protein) muc (staphylococcal nuclease) seb (enterotoxin type B precursor) seg (staphylococcal enterotoxin G)	Staphylococcus aureus Staphylococcus	49482253 49482253 49482253 49482253 49482253 49482253 49484912 49489772 52698233 57634611 57634611
BLAZ NC002952 ERMA_NC002952 ERMB_Y13600 SEA-SEE_NC002952 SEA_SEE_NC002952 SEE_NC002952 SEE_NC002953 ERMC_NC002953 ERMC_NC002953 ERMC_NC002953 ERMC_NC002953 SEE_NC002958 SEE_NC002958 SEE_NC002758 SEE_NC002758 SEE_NC002758	blaz (beta lactamase III) ermà (IRNA methyltransferase A) erma (IRNA methyltransferase B) erma (IRNA methyltransferase B) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor) erma (IRNA methyltransferase C) erma (IRNA methyltransferase C) muts (DNA minustch repair protein) muc (staphylococcal enterotoxin H) seb (enterotoxin type B precursor) seb (enterotoxin type B precursor) seg (staphylococcal enterotoxin G) sei (staphylococcal enterotoxin I)	Върру/ососия виченя Върру/ососия	49482253 49482253 49482253 49482253 49482253 49482253 49482253 49489772 52698233 57634611 57634611 57634611
BLAE_NC002952 ERMA_NC002952 ERMB_Y13600 SEA-SEE_NC002952 SEE_NC002952 SEE_NC002952 SEE_NC002952 SEE_NC002953 ERMC_NC005968 MUTS_A74598802 NUC_NC002758 SEB_NC002758 SEB_NC002758	blaz (beta lactamase III) ermA (rENA methyltransferase A) ermS (rENA methyltransferase B) sea (staphylococcal enterotoxin A resea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin B ermC (rENA methyltransferase C) muts (DNA miswatch repair protein) muc (staphylococcal nuclease) seb (enterotoxin type B precursor) seg (staphylococcal enterotoxin G)	Staphylococcus sureus Staphylococcus sureus sureus sureus sureus sureus sureus sureus sureus sureus staphylococcus sureus staphylococcus sureus staphylococcus sureus staphylococcus sureus sureus sureus sureus sureus staphylococcus staphylococcus staphylococcus sureus sureus	49482253 49482253 49482253 49482253 49482253 49482253 49484912 49489772 52698233 57634611 57634611
BLAZ NC002952 ERMA_NC002952 ERMB_Y13600 SEA-SEE_NC002952 SEA_SEE_NC002952 SEE_NC002952 SEE_NC002953 ERMC_NC002953 ERMC_NC002953 ERMC_NC002953 ERMC_NC002953 SEE_NC002958 SEE_NC002958 SEE_NC002758 SEE_NC002758 SEE_NC002758	blaz (beta lactamase III) ermà (IRNA methyltransferase A) erma (IRNA methyltransferase B) erma (IRNA methyltransferase B) sea (staphylococcal enterotoxin A precursor) sea (staphylococcal enterotoxin A precursor) erma (IRNA methyltransferase C) erma (IRNA methyltransferase C) muts (DNA minustch repair protein) muc (staphylococcal enterotoxin H) seb (enterotoxin type B precursor) seb (enterotoxin type B precursor) seg (staphylococcal enterotoxin G) sei (staphylococcal enterotoxin I)	Върру/ососия виченя Върру/ососия	49482253 49482253 49482253 49482253 49482253 49482253 49482253 49489772 52698233 57634611 57634611 57634611

[373] Note: artificial reference sequences represent concatenations of partial gene extractions from the indicated reference gi number. Partial sequences were used to create the concatenated sequence because complete gene sequences were not necessary for primer design.

Example 2: Sample Preparation and PCR

[374] Genomic DNA was prepared from samples using the DNeasy Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's protocols.

[375] All PCR reactions were assembled in 50 µL reaction volumes in a 96-well microtiter plate format using a Packard MPII liquid handling robotic platform and M.J. Dyad thermocyclers (MJ research, Waltham, MA) or Eppendorf Mastercycler thermocyclers (Eppendorf, Westbury, NY). The PCR reaction mixture consisted of 4 units of Amplitaq Gold, 1x buffer II (Applied Biosystems, Foster City, CA), 1.5 mM MgCl₂, 0.4 M betaine, 800 µM dNTP mixture and 250 nM of each primer. The following typical PCR conditions were used: 95°C for 10 min followed by 8 cycles of 95°C for 30 seconds, 48°C for 30 seconds, and 72°C 30 seconds with the 48°C annealing temperature increasing 0.9°C with each of the eight cycles. The PCR was then continued for 37 additional cycles of 95°C for 15 seconds, 56°C for 20 seconds. and 72°C 20 seconds.

Example 3: Purification of PCR Products for Mass Spectrometry with Ion Exchange Resin-Magnetic Beads

[376] For solution capture of nucleic acids with ion exchange resin linked to magnetic beads, $25~\mu$ l of a 2.5 mg/mL suspension of BioClone amine terminated superparamagnetic beads were added to 25 to 50 μ l of a PCR (or RT-PCR) reaction containing approximately 10 pM of a typical PCR amplification product. The above suspension was mixed for approximately 5 minutes by vortexing or pipetting, after which the liquid was removed after using a magnetic separator. The beads containing bound PCR amplification product were then washed three times with 50mM armonium bicarbonate/50% MeOH or 100mM ammonium bicarbonate/50% MeOH, followed by three more washes with 50% MeOH. The bound PCR amplicon was cluted with a solution of 25mM piperidine, 25mM imidazole, 35% MeOH which included peptide calibration standards.

Example 4: Mass Spectrometry and Base Composition Analysis

[377] The ESI-FTICR mass spectrometer is based on a Bruker Daltonics (Billerica, MA) Apex II

70e electrospray ionization Fourier transform ion cyclotron resonance mass spectrometer that employs
an actively shielded 7 Tesla superconducting magnet. The active shielding constrains the majority of the
fringing magnetic field from the superconducting magnet to a relatively small volume. Thus,

components that might be adversely affected by stray magnetic fields, such as CRT monitors, robotic components, and other electronics, can operate in close proximity to the FTICR spectrometer. All aspects of pulse sequence control and data acquisition were performed on a 600 MHz Pentium II data station running Bruker's Xmass software under Windows NT 4.0 operating system. Sample aliquots, typically 15 ul. were extracted directly from 96-well microtiter plates using a CTC HTS PAL autosampler (LEAP Technologies, Carrboro, NC) triggered by the FTICR data station. Samples were injected directly into a 10 µl sample loop integrated with a fluidics handling system that supplies the 100 ul /hr flow rate to the ESI source. Ions were formed via electrospray ionization in a modified Analytica (Branford, CT) source employing an off axis, grounded electrospray probe positioned approximately 1.5 cm from the metalized terminus of a glass desolvation capillary. The atmospheric pressure end of the glass capillary was biased at 6000 V relative to the ESI needle during data acquisition. A countercurrent flow of dry N2 was employed to assist in the desolvation process. Ions were accumulated in an external ion reservoir comprised of an rf-only hexapole, a skimmer cone, and an auxiliary gate electrode, prior to injection into the trapped ion cell where they were mass analyzed. Ionization duty cycles greater than 99% were achieved by simultaneously accumulating ions in the external ion reservoir during ion detection. Each detection event consisted of 1M data points digitized over 2.3 s. To improve the signalto-noise ratio (S/N), 32 scans were co-added for a total data acquisition time of 74 s.

[378] The ESI-TOF mass spectrometer is based on a Bruker Daltonics MicroTOFTM. Ions from the ESI source undergo orthogonal ion extraction and are focused in a reflectron prior to detection. The TOF and FTICR are equipped with the same automated sample handling and fluidios described above. Ions are formed in the standard MicroTOFTM ESI source that is equipped with the same off-axis sprayer and glass capillary as the FTICR ESI source. Consequently, source conditions were the same as those described above. External ion accumulation was also employed to improve ionization duty cycle during data acquisition. Each detection event on the TOF was comprised of 75,000 data points digitized over 75 µs.

[379] The sample delivery scheme allows sample aliquots to be rapidly injected into the electrospray source at high flow rate and subsequently be electrosprayed at a much lower flow rate for improved ESI sensitivity. Prior to injecting a sample, a bolus of buffer was injected at a high flow rate to rinse the transfer line and spray needle to avoid sample contamination/carryover. Following the rinse step, the autosampler injected the next sample and the flow rate was switched to low flow. Following a brief equilibration delay, data acquisition commenced. As spectra were co-added, the autosampler continued rinsing the syringe and picking up buffer to rinse the injector and sample transfer line. In general, two syringe rinses and one injector rinse were required to minimize sample carryover. During a

routine screening protocol a new sample mixture was injected every 106 seconds. More recently a fast wash station for the syringe needle has been implemented which, when combined with shorter acquisition times, facilitates the acquisition of mass spectra at a rate of just under one spectrum/minute.

[380] Raw mass spectra were post-calibrated with an internal mass standard and deconvoluted to monoisotopic molecular masses. Unambiguous base compositions were derived from the exact mass measurements of the complementary single-stranded oligonucleotides. Quantitative results are obtained by comparing the peak heights with an internal PCR calibration standard present in every PCR well at 500 molecules per well. Calibration methods are commonly owned and disclosed in U.S. Provisional Patent Application Serial No. 60/545,425 which is incorporated herein by reference in entirety.

Example 5: De Novo Determination of Base Composition of Amplification Products using Molecular Mass Modified Deoxynucleotide Triphosphates

- [381] Because the molecular masses of the four natural nucleobases have a relatively narrow molecular mass range (A = 313.058, G = 329.052, C = 289.046, T = 304.046 See Table 4), a persistent source of ambiguity in assignment of base composition can occur as follows: two nucleic acid strands having different base composition may have a difference of about 1 Da when the base composition difference between the two strands is $G \leftrightarrow A$ (-15.994) combined with $C \leftrightarrow T$ (+15.000). For example, one 99-mer nucleic acid strand having a base composition of $A_{27}G_{30}C_{31}T_{21}$ has a theoretical molecular mass of 30779.058 while another 99-mer nucleic acid strand having a base composition of $A_{26}G_{31}C_{22}T_{20}$ has a theoretical molecular mass of 30780.052. A 1 Da difference in molecular mass may be within the experimental error of a molecular mass measurement and thus, the relatively narrow molecular mass range of the four natural nucleobases imposes an uncertainty factor.
- [382] The present invention provides for a means for removing this theoretical 1 Da uncertainty factor through amplification of a nucleic acid with one mass-tagged nucleobase and three natural nucleobases. The term "nucleobase" as used herein is synonymous with other terms in use in the art including "nucleotide," "deoxynucleotide," "nucleotide residue," "deoxynucleotide residue," "nucleotide triphosobate (NTP)." or deoxynucleotide triphosobate (NTP).
- [383] Addition of significant mass to one of the 4 nucleobases (dNTPs) in an amplification reaction, or in the primers themselves, will result in a significant difference in mass of the resulting amplification product (significantly greater than 1 Da) arising from ambiguities mising from the G ↔ A combined with C ↔ T event (Table 4). Thus, the same the G ↔ A (-15.994) event combined with 5-lodo-C ↔ T (-110.900) event would result in a molecular mass difference of 126.894. If the molecular mass of the

base composition $A_{27}G_{30}$ 5-Iodo- $C_{21}T_{21}$ (33422.958) is compared with $A_{26}G_{31}$ 5-Iodo- $C_{27}T_{20}$, (33549.852) the theoretical molecular mass difference is +126.894. The experimental error of a molecular mass measurement is not significant with regard to this molecular mass difference. Furthermore, the only base composition consistent with a measured molecular mass of the 99-mer nucleic acid is $A_{27}G_{30}$ 5-Iodo- $C_{21}T_{21}$. In contrast, the analogous amplification without the mass tag has 18 possible base compositions.

Table 4: Molecular Masses of Natural Nucleobases and the Mass-Modified Nucleobase 5-Jodo-C
and Molecular Mass Differences Resulting from Transitions

Nucleobase	Molecular Mass	Transition	Δ Molecular Mass
A	313.058	A>T	-9.012
A	313.058	A>C	-24.012
A	313.058	A>5-Iodo-C	101.888
A	313.058	A>G	15.994
T	304.046	T>A	9.012
T	304.046	T>C	-15.000
т	304.046	T>5-Iodo-C	110.900
Т	304.046	T>G	25.006
С	289.046	C>A	24.012
С	289.046	C>T	15.000
С	289.046	C>G	40.006
5-Iodo-C	414.946	5-Iodo-C>A	-101.888
5-Iodo-C	414.946	5-Iodo-C>T	-110.900
5-Iodo-C	414.946	5-Iodo-C>G	-85.894
G	329.052	G>A	-15.994
G	329.052	G>T	-25.006
G	329.052	G>C	-40.006
G	329.052	G>5-Iodo-C	85.894

[384] Mass spectra of bioagent-identifying amplicons were analyzed independently using a maximum-likelihood processor, such as is widely used in radar signal processing. This processor, referred to as GenX, first makes maximum likelihood estimates of the input to the mass spectrometer for each primer by running matched filters for each base composition aggregate on the input data. This includes the GenX response to a calibrant for each primer.

[385] The algorithm emphasizes performance predictions culminating in probability-of-detection versus probability-of-false-alarm plots for conditions involving complex backgrounds of naturally occurring organisms and environmental contaminants. Matched filters consist of a priori expectations of signal values given the set of primers used for each of the bioagents. A genomic sequence database is

used to define the mass base count matched filters. The database contains the sequences of known bacterial bioagents and includes threat organisms as well as benign background organisms. The latter is used to estimate and subtract the spectral signature produced by the background organisms. A maximum likelihood detection of known background organisms is implemented using matched filters and a running-sum estimate of the noise covariance. Background signal strengths are estimated and used along with the matched filters to form signatures which are then subtracted. The maximum likelihood process is applied to this "cleaned up" data in a similar manner employing matched filters for the organisms and a running-sum estimate of the noise-covariance for the cleaned up data.

[386] The amplitudes of all base compositions of bioagent-identifying amplicons for each primer are calibrated and a final maximum likelihood amplitude estimate per organism is made based upon the multiple single primer estimates. Models of all system noise are factored into this two-stage maximum likelihood calculation. The processor reports the number of molecules of each base composition contained in the spectra. The quantity of amplification product corresponding to the appropriate primer set is reported as well as the quantities of primers remaining upon completion of the amplification reaction.

1387] Base count blurring can be carried out as follows. "Electronic PCR" can be conducted on nucleotide sequences of the desired bioagents to obtain the different expected base counts that could be obtained for each primer pair. See for example, nobi.nlm.nih.gov/sutils/e-pcr/; Schuler, Genome Res. 7:541-50, 1997. In one illustrative embodiment, one or more spreadsbeets, such as Microsoft Excel workbooks contain a plurality of worksheets. First in this example, there is a worksheet with a name similar to the workbook name; this worksheet contains the raw electronic PCR data. Second, there is a worksheet named "filtered bioagents base count" that contains bioagent name and base count; there is a separate record for each strain after removing sequences that are not identified with a genus and species and removing all sequences for bioagents with less than 10 strains. Third, there is a worksheet, "Sheet1" that contains the frequency of substitutions, insertions, or deletions for this primer pair. This data is generated by first creating a pivot table from the data in the "filtered bioagents base count" worksheet and then executing an Excel VBA macro. The macro creates a table of differences in base counts for bioagents of the same species, but different strains. One of ordinary skill in the art may understand additional pathways for obtaining similar table differences without undo experimentation.

[388] Application of an exemplary script, involves the user defining a threshold that specifies the fraction of the strains that are represented by the reference set of base counts for each bioagent. The reference set of base counts for each bioagent may contain as many different base counts as are needed

to meet or exceed the threshold. The set of reference base counts is defined by taking the most abundant strain's base type composition and adding it to the reference set and then the next most abundant strain's base type composition is added until the threshold is met or exceeded. The current set of data was obtained using a threshold of 55%, which was obtained empirically.

[389] For each base count not included in the reference base count set for that bioagent, the script then proceeds to determine the manner in which the current base count differs from each of the base counts in the reference set. This difference may be represented as a combination of substitutions, Si=Xi, and insertions, Ii=Yi, or deletions, Di=Zi. If there is more than one reference base count, then the reported difference is chosen using rules that aim to minimize the number of changes and, in instances with the same number of changes, minimize the number of insertions or deletions. Therefore, the primary rule is to identify the difference with the minimum sum (Xi+Yi) or (Xi+Zi), e.g., one insertion rather than two substitutions. If there are two or more differences with the minimum sum, then the one that will be reported is the one that contains the most substitutions.

[390] Differences between a base count and a reference composition are categorized as one, two, or more substitutions, one, two, or more insertions, one, two, or more deletions, and combinations of substitutions and insertions or deletions. The different classes of nucleobase changes and their probabilities of occurrence have been delineated in U.S. Patent Application Publication No. 2004209260 (U.S. Application Serial No. 10/418,514) which is incorporated herein by reference in entirety.

Example 6: Use of Broad Range Survey and Division Wide Primer Pairs for Identification of Bacteria in an Epidemic Surveillance Investigation

[391] This investigation employed a set of 16 primer pairs which is herein designated the
"surveillance primer set" and comprises broad range survey primer pairs, division wide primer pairs and a single Bacillus clade primer pair. The surveillance primer set is shown in Table 5 and consists of
primer pairs originally listed in Table 2. This surveillance set comprises primers with T modifications
(note TMOD designation in primer names) which constitutes a functional improvement with regard to
prevention of non-templated adenylation (vide supra) relative to originally selected primers which are
displayed below in the same row. Primer pair 449 (non-T modified) has been modified twice. Its
predecessors are primer pairs 70 and 357, displayed below in the same row. Primer pair 360 has also
been modified twice and its predecessors are primer pairs 17 and 118.

Table 5: Bacterial Primer Pairs of the Surveillance Primer Set

Primer Forward Primer Name	Forward Primer	Reverse Primer Name	Reverse Primer	Target Gene	

No.		(SEQ ID NO:)		(SEQ ID NO:)	
346	16S_EC_713_732_TMOD_F	202	16S_EC_789_809_TMOD_R	1110	16S rRNA
10	16S_EC_713_732_F	21	168_EC_789_809	798	16S rRNA
347	16S_EC_785_806_TMOD_F	560	16S_EC_880_897_TWOD_R	1278	16S TRNA
11	16S_EC_785_806_F	118	16S_BC_880_897_R	830	16S TRNA
348	16S_EC_960_981_TWOD_F	706	16S_EC_1054_1073_TWOD_R	895	16S TRNA
14	16S EC 960 981 F	672	16S EC 1054 1073 R	735	168 rRNA
349	23S_EC_1826_1843_TMOD_F	401	23S_EC_1906_1924_TWOD_R	1156	23S rRNA
16	23S_EC_1826_1843_F	80	23S_EC_1906_1924_R	805	238 rRNA
352	INFB_EC_1365_1393_TMOD_F	687	INFB_EC_1439_1467_TMOD_R	1411	infB
34	INFB BC 1365 1393 F	524	INFB EC 1439 1467 R	1248	infB
354	RPOC_EC_2218_2241_TWOD_F	405	RPOC_EC_2313_2337_TNOD_R	1072	rpoC
52	RPOC_EC_2218_2241_F	81	RPOC_EC_2313_2337_R	790	rpoC
355	SSPE_BA_115_137_TMOD_F	255	SSPE_BA_197_222_TMOD_R	1402	sapE
58	SSPE_BA_115_137_F	45	SSPE BA 197 222 R	1201	sspE
356	RPLB_BC_650_679_TMOD_F	232	RPLB_EC_739_762_TMOD_R	592	rplB
66	RPLB_EC_650_679_F	98	RPLB_EC_739_762_R	999	rplB
358	VALS_EC_1105_1124_TMOD_F	385	VALS_EC_1195_1218_TNOD_R	1093	valS
71	VALS_EC_1105_1124_F	77	VALS_EC_1195_1218_R	795	vals
359	RPOB_EC_1845_1866_TWOD_F	659	RPOB_EC_1909_1929_TMOD_R	1250	rpoB
72	RPOB_EC_1845_1866_F	233	RPOB_EC_1909_1929_R	825	rpoB
360	23S_EC_2646_2667_TMOD_F	409	23S_EC_2745_2765_TMOD_R	1434	23S TRNA
118	238_EC_2646_2667_F	84 .	23S_EC_2745_2765_R	1389	238 rRNA
17	23S_EC_2645_2669_F	408	23S_EC_2744_2761_R	1252	23S TRNA
361	168_EC_1090_1111_2_TMOD_F	697	16S_EC_1175_1196_TWOD_R	1398	16S TRNA
3	16S EC 1090 1111 2 F	651	16S EC 1175 1196 R	1159	16S TRNA
362	RPOB_EC_3799_3821_TMOD_F	581	RPOB_EC_3862_3888_TMOD_R	1325	rpoB
289	RPOB_EC_3799_3821_F	124	RPOB_EC_3862_3888_R	840	rpoB
363	RPOC_EC_2146_2174_TMOD_F	284	RPOC_EC_2227_2245_TNOD_R	898	rpoC
290	RPOC_EC_2146_2174_F	52	RPOC_EC_2227_2245_R	736	rpoC
367	TUFB_EC_957_979_TWOD_F	308	TUFB_EC_1034_1058_TNOD_R	1276	tufB
293	TUFB_EC_957_979_F	55	TUFB EC 1034 1058 R	829	tufB
449	RPLB_EC_690_710_F	309	RPLB_EC_737_758_R	1336	rplB
357	RPLB_EC_688_710_TMOD_F	296	RPLB_EC_736_757_TMOD_R	1337	rplB
67	RPLB EC 688 710 F	54	RPLB_EC_736_757_R	842	rplB

[392] The 16 primer pairs of the surveillance set are used to produce bioagent identifying amplicons whose base compositions are sufficiently different amongst all known bacteria at the species level to identify, at a reasonable confidence level, any given bacterium at the species level. As shown in Tables 6A-E, common respiratory bacterial pathogens can be distinguished by the base compositions of bioagent identifying amplicons obtained using the 16 primer pairs of the surveillance set. In some cases, triangulation identification improves the confidence level for species assignment. For example, nucleic acid from Streptococcus progenes can be amplified by nine of the sixteen surveillance primer pairs and Streptococcus preumoniae can be amplified by ten of the sixteen surveillance primer pairs. The base

compositions of the bioagent identifying amplicons are identical for only one of the analogous bioagent identifying amplicons and differ in all of the remaining analogous bioagent identifying amplicons by up to four bases per bioagent identifying amplicon. The resolving power of the surveillance set was confirmed by determination of base compositions for 120 isolates of respiratory pathogens representing 70 different bacterial species and the results indicated that natural variations (usually only one or two base substitutions per bioagent identifying amplicon) amongst multiple isolates of the same species did not prevent correct identification of major pathogenic organisms at the species level.

[393] Bacillus anthracis is a well known biological warfare agent which has emerged in domestic terrorism in recent years. Since it was envisioned to produce bioagent identifying amplicons for identification of Bacillus anthracis, additional drill-down analysis primers were designed to target genes present on virulence plasmids of Bacillus anthracis so that additional confidence could be reached in positive identification of this pathogenic organism. Three drill-down analysis primers were designed and are listed in Tables 2 and 6. In Table 6, the drill-down set comprises primers with T modifications (note TMOD designation in primer names) which constitutes a functional improvement with regard to prevention of non-templated adenylation (vide supra) relative to originally selected primers which are displayed below in the same row.

Table 6: Drill-Down Primer Pairs for Confirmation of Identification of Bacillus anthracis

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
350	CAPC_BA_274_303_TMOD_F	476	CAPC_BA_349_376_TMOD_R	1314	capC
24	CAPC_BA_274_303_F	109	CAPC BA 349 376 R	837	CapC
351	CYA_BA_1353_1379_TMOD_F	355	CYA_BA_1448_1467_TMOD_R	1423	cyA
30	CYA BA 1353 1379 F	64	CYA BA 1448 1467 R	1342	CYA
353	LEF_BA_756_781_TMOD_F	220	LEF_BA_843_872_TMOD_R	1394	lef
37	LEF_BA_756_781_F	26	LEF_BA_843 872 R	1135	lef

[394] Phylogenetic coverage of bacterial space of the sixteen surveillance primers of Table 5 and the three Bacillus anthracis drill-down primers of Table 6 is shown in Figure 3 which lists common pathogenic bacteria. Figure 3 is not meant to be comprehensive in illustrating all species identified by the primers. Only pathogenic bacteria are listed as representative examples of the bacterial species that can be identified by the primers and methods of the present invention. Nucleic acid of groups of bacteria enclosed within the polygons of Figure 3 can be amplified to obtain bioagent identifying amplicons using the primer pair numbers listed in the upper right hand corner of each polygon. Primer coverage for polygons within polygons is additive. As an illustrative example, bioagent identifying amplicons can be obtained for Chlamydia trachomatis by amplification with, for example, primer pairs 346-349, 360 and

361, but not with any of the remaining primers of the surveillance primer set. On the other hand, bioagent identifying amplicons can be obtained from nucleic acid originating from Bacillus anthracis (located within 5 successive polygons) using, for example, any of the following primer pairs: 346-349, 360, 361 (base polygon), 356, 449 (second polygon), 352 (third polygon), 355 (fourth polygon), 350, 351 and 353 (fifth polygon). Multiple coverage of a given organism with multiple primers provides for increased confidence level in identification of the organism as a result of enabling broad triangulation identification.

[395] In Tables 7A-E, base compositions of respiratory pathogens for primer target regions are shown. Two entries in a cell, represent variation in ribosomal DNA operons. The most predominant base composition is shown first and the minor (frequently a single operon) is indicated by an asterisk (*). Entries with NO DATA mean that the primer would not be expected to prime this species due to mismatches between the primer and target region, as determined by theoretical PCR.

Table 7A – Base Compositions of Common Respiratory Pathogens for Bioagent Identifying

Amplicons Corresponding to Primer Pair Nos: 346, 347 and 348

		Primer 346	Primer 347	Primer 348
Organism	Strain	[AGCT]	[AGCT]	[AGCT]
Klebsiella		[29 32 25 13]	[23 38 28 26]	[26 32 28 30]
pneumoniae	MGH78578	[29 31 25 13]*	[23 37 28 26]*	[26 31 28 30]*
	CO-92 Biovar			[29 30 28 29]
Yersinia pestis	Orientalis	[29 32 25 13]	[22 39 28 26]	[30 30 27 29]*
	KIM5 P12 (Biovar			
Yersinia pestis	Mediaevalis)	[29 32 25 13]	[22 39 28 26]	[29 30 28 29]
				[29 30 28 29]
Yersinia pestis	91001	[29 32 25 13]	[22 39 28 26]	[30 30 27 29]*
Haemophilus				
influenzae	KW20	[28 31 23 17]	[24 37 25 27]	[29 30 28 29]
Pseudomonas			[26 36 29 24]	1
aeruginosa	PA01	[30 31 23 15]	[27 36 29 23]*	[26 32 29 29]
Pseudomonas				ı
fluorescens	P£0-1	[30 31 23 15]	[26 35 29 25]	[28 31 28 29]
Pseudomonas	1		l .	1
putida	KT2440	[30 31 23 15]	[28 33 27 27]	[27 32 29 28]
Legionella				
pneumophila	Philadelphia-1	[30 30 24 15]	[33 33 23 27]	[29 28 28 31]
Francisella		1		
tularensis	schu 4	[32 29 22 16]	[28 38 26 26]	[25 32 28 31]
Bordetella	i .			ı
pertussis	Tohama I	[30 29 24 16]	[23 37 30 24]	[30 32 30 26]
Burkholderia	1			[27 36 31 24]
cepacia	J2315	[29 29 27 14]	[27 32 26 29]	[20 42 35 19]*
Burkholderia		l .		1
pseudomallei	K96243	[29 29 27 14]	[27 32 26 29]	[27 36 31 24]
Neisseria	FA 1090, ATCC			
gonorrhoeae	700825	[29 28 24 18]	[27 34 26 28]	[24 36 29 27]
Neisseria	l			
meningitidis	MC58 (serogroup B)	[29 28 26 16]	[27 34 27 27]	[25 35 30 26]
Neisseria				
meningitidis	serogroup C, FAM18	[29 28 26 16]	[27 34 27 27]	[25 35 30 26]
Neisseria				
meningitidis	Z2491 (serogroup A)	[29 28 26 16]	[27 34 27 27]	[25 35 30 26]
Chlamydophila			l	
pneumoniae	TW-183	[31 27 22 19]	NO DATA	[32 27 27 29]

Chlamydophila				
pneumoniae	AR39	[31 27 22 19]	NO DATA	[32 27 27 29]
Chlamydophila				
pneumoniae	CMT039	[31 27 22 19]	NO DATA	[32 27 27 29]
Chlamydophila				
pneumoniae	J138	[31 27 22 19]	NO DATA	[32 27 27 29]
Corynebacterium		Į.		
diphtheriae	NCTC13129	[29 34 21 15]	[22 38 31 25]	[22 33 25 34]
Mycobacterium				
avium	k10	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
Mycobacterium		i	l	
avium	104	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
Mycobacterium			l .	
tuberculosis	CSU#93	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
Mycobacterium	i			
tuberculosis	CDC 1551	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
Mycobacterium				1
tuberculosis	H37Rv (lab strain)	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
Mycoplasma				
pneumoniae	M129	[31 29 19 20]	NO DATA	NO DATA
Staphylococcus				[30 29 30 29]
aureus	MRSA252	[27 30 21 21]	[25 35 30 26]	[29 31 30 29]*
Staphylococcus	l			[30 29 30 29]
aureus	MSSA476	[27 30 21 21]	[25 35 30 26]	[30 29 29 30]*
Staphylococcus				[30 29 30 29]
aureus	COF	[27 30 21 21]	[25 35 30 26]	[30 29 29 30]*
Staphylococcus				[30 29 30 29]
aureus	Mu50	[27 30 21 21]	[25 35 30 26]	[30 29 29 30]*
Staphylococcus			l	[30 29 30 29]
aureus	MW2	[27 30 21 21]	[25 35 30 26]	[30 29 29 30]*
Staphylococcus	l			[30 29 30 29]
aureus	N315	[27 30 21 21]	[25 35 30 26]	[30 29 29 30]*
Staphylococcus			[25 35 30 26]	[30 29 30 29]
aureus	NCTC 8325	[27 30 21 21]	[25 35 31 26]*	[30 29 29 30]
Streptococcus			[24 36 31 25]	
agalactiae	NEM316	[26 32 23 18]	[24 36 30 26]*	[25 32 29 30]
Streptococcus	_			1
equi	NC_002955	[26 32 23 18]	[23 37 31 25]	[29 30 25 32]
Streptococcus				
pyogenes	MGAS8232	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
Streptococcus				
pyogenes	MGAS315	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
Streptococcus				
pyogenes	SSI-1	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
Streptococcus		/		ran as as as
pyogenes	MGAS10394	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
Streptococcus	V			
pyogenes	Manfredo (M5)	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
Streptococcus	amama (sea.)	/	for an an an	fam as as as
pyogenes	SF370 (M1)	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
Streptococcus	670	for 20 00 401	for ar on onl	for 20 00 202
pneumoniae	670	[26 32 23 18]	[25 35 28 28]	[25 32 29 30]
Streptococcus pneumoniae	R6	1 000 00 00 001	for ar on onl	for 20 00 001
	Rb	[26 32 23 18]	[25 35 28 28]	[25 32 29 30]
Streptococcus pneumoniae	TIGR4	126 22 22 101	for 25 00 003	top 20 20 201
	TIGRA	[26 32 23 18]	[25 35 28 28]	[25 32 30 29]
Streptococcus gordonii	NCTC7868	for 22 22 101	104 25 21 251	for 21 20 211
	NC1C/868	[25 33 23 18]	[24 36 31 25]	[25 31 29 31]
Streptococcus mitis	L waren 1	1	tor or oc.	[25 32 29 30]
Mitis Streptococcus	NCTC 12261	[26 32 23 18]	[25 35 30 26]	[24 31 35 29]*
Streptococcus mutans	UA159	1 104 00 04 100	[25 37 30 24]	100 21 05 241
mucans	OWTOA	[24 32 24 19]	[45 37 30 24]	[28 31 26 31]

Table 7B - Base Compositions of Common Respiratory Pathogens for Bioagent Identifying
Amplicons Corresponding to Primer Pair Nos: 349, 360, and 356

	Strain		Primer 360	Primer 356
Organism		Primer 349		Primer 356

		[AGCT]	[A G C T]	[AGCT]
Klebsiella				
pneumoniae	MGH78578	[25 31 25 22]	[33 37 25 27]	NO DATA
	CO-92 Biovar	[25 31 27 20]		
Yersinia pestis	Orientalis	[25 32 26 20]*	[34 35 25 28]	NO DATA
	KIMS P12 (Biovar	[25 31 27 20]		
Yersinia pestis	Mediaevalis)	[25 32 26 20]*	[34 35 25 28]	NO DATA
Yersinia pestis	91001	[25 31 27 20]	[34 35 25 28]	NO DATA
Haemophilus		1		
influenzae	KW20	[28 28 25 20]	[32 38 25 27]	NO DATA
Pseudomonas	KHEU	[20 20 25 20]	[31 36 27 27]	NO DITTA
aeruginosa	PAO1	[24 31 26 20]	[31 36 27 28]*	NO DATA
Pseudomonas	PAUL	[24 31 26 20]	[30 37 27 28]	NU DATA
fluorescens	Pf0-1	NO DATA	[30 37 27 28]	NO DATA
Pseudomonas	PIU-I	NO DATA	[30 37 27 20]	NO DATA
	l		/	
putida	KT2440	[24 31 26 20]	[30 37 27 28]	NO DATA
Legionella				
pneumophila	Philadelphia-1	[23 30 25 23]	[30 39 29 24]	NO DATA
Francisella				
tularensis	schu 4	[26 31 25 19]	[32 36 27 27]	NO DATA
Bordetella				
pertussis	Tohama I	[21 29 24 18]	[33 36 26 27]	NO DATA
Burkholderia				
cepacia	J2315	[23 27 22 20]	[31 37 28 26]	NO DATA
Burkholderia				
pseudomallei	K96243	[23 27 22 20]	[31 37 28 26]	NO DATA
Neisseria				
gonorrhoeae	FA 1090, ATCC 700825	[24 27 24 17]	[34 37 25 26]	NO DATA
Neisseria	, , , , , , , , , , , , , , , , , , , ,			
meningitidis	MC58 (serogroup B)	[25 27 22 18]	[34 37 25 26]	NO DATA
Neisseria	(,		
meningitidis	serogroup C, FAMLS	[25 26 23 18]	[34 37 25 26]	NO DATA
Neisseria	Surugioup C, Think	[25 20 25 20]	[54 57 25 26]	No Billin
meningitidis	Z2491 (serogroup A)	[25 26 23 18]	[34 37 25 26]	NO DATA
Chlamydophila	Entre (Scrogroup II)	[25 26 25 26]	[54 57 25 25]	No Billi
pneumoniae	TW-183	[30 28 27 18]	NO DATA	NO DATA
Chlamydophila	1M-193	[30 20 27 10]	NO DATA	NO DATA
pneumoniae	AR39	[30 28 27 18]	NO DATA	NO DATA
Chlamydophila	ARSS	[30 20 27 10]	NO DATA	NO DATA
pneumoniae	CWL029	[30 28 27 18]	NO DATA	NO DATA
	CWL029	[30 28 27 18]	NO DATA	NO DATA
Chlamydophila				1
pneumoniae	J138	[30 28 27 18]	NO DATA	NO DATA
Corynebacterium				
diphtheriae	NCTC13129	NO DATA	[29 40 28 25]	NO DATA
Mycobacterium			1 -	l
avium	k10	NO DATA	[33 35 32 22]	NO DATA
Mycobacterium			1.	
avium	104	NO DATA	[33 35 32 22]	NO DATA
Mycobacterium				
tuberculosis	CSU#93	NO DATA	[30 36 34 22]	NO DATA
Mycobacterium			1	
tuberculosis	CDC 1551	NO DATA	[30 36 34 22]	NO DATA
Mycobacterium				
tuberculosis	H37Rv (lab strain)	NO DATA	[30 36 34 22]	NO DATA
Mycoplasma	ľ			
pneumoniae	M129	[28 30 24 19]	[34 31 29 28]	NO DATA
Staphylococcus		Ţ		
aureus	MRSA252	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
Staphylococcus				
aureus	MSSA476	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
Staphylococcus				· ·
aureus	COF	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
Staphylococcus		T		· · · · · · · · · · · · · · · · · · ·
aureus	Mu50	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
Staphylococcus				
aureus	NW2	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
Staphylococcus		30 25 25		
aureus	N315	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
Staphylococcus	NCTC 8325	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]

aureus	T			T
Streptococcus				
agalactiae	NEM316	[28 31 22 20]	[33 37 24 28]	[37 30 28 26]
Streptococcus				
equi	NC 002955	[28 31 23 19]	[33 38 24 27]	[37 31 28 25]
Streptococcus				
pyogenes	MGASB232	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
Streptococcus				
pyogenes	MGAS315	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
Streptococcus				
pyogenes	SSI-1	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
Streptococcus				
pyogenes	MGAS10394	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
Streptococcus	1	[28 31 23 19]	fan an at an	(00 00 00 00)
pyogenes	Manfredo (M5)	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
Streptococcus pyogenes	SF370 (M1)	[28 31 22 20]*	[33 37 24 28]	[38 31 29 23]
Streptococcus	SF3/0 (MI)	[26 31 22 20]-	[33 37 24 20]	[30 31 29 23]
pneumoniae	670	[28 31 22 20]	[34 36 24 28]	[37 30 29 25]
Streptococcus		(20 02 02 00)	101 00 01 007	101 00 01 007
pneumoniae	R6	[28 31 22 20]	[34 36 24 28]	[37 30 29 25]
Streptococcus				·
pneumoniae	TIGR4	[28 31 22 20]	[34 36 24 28]	[37 30 29 25]
Streptococcus				
gordonii	NCTC7868	[28 32 23 20]	[34 36 24 28]	[36 31 29 25]
Streptococcus		[28 31 22 20]		
mitis	NCTC 12261	[29 30 22 20]*	[34 36 24 28]	[37 30 29 25]
Streptococcus				
mutans	UA159	[26 32 23 22]	[34 37 24 27]	NO DATA

Table 7C – Base Compositions of Common Respiratory Pathogens for Bioagent Identifying
Amplicons Corresponding to Primer Pair Nos: 449, 354, and 352

		Primer 449	Primer 354	Primer 352
Organism	Strain	[AGCT]	[AGCT]	[AGCT]
Klebsiella				
pneumoniae	MGH78578	NO DATA	[27 33 36 26]	NO DATA
	CO-92 Biovar			
Yersinia pestis	Orientalis	NO DATA	[29 31 33 29]	[32 28 20 25]
	KIMS P12 (Biovar			
Yersinia pestis	Mediaevalis)	NO DATA	[29 31 33 29]	[32 28 20 25]
Yersinia pestis	91001	NO DATA	[29 31 33 29]	NO DATA
Haemophilus				
influenzae	KW20	NO DATA	[30 29 31 32]	NO DATA
Pseudomonas				
aeruginosa	PAO1	NO DATA	[26 33 39 24]	NO DATA
Pseudomonas				
fluorescens	Pf0-1	NO DATA	[26 33 34 29]	NO DATA
Pseudomonas		1		
putida	KT2440	NO DATA	[25 34 36 27]	NO DATA
Legionella				
pneumophila	Philadelphia-1	NO DATA	NO DATA	NO DATA
Francisella		i e		1
tularensis	schu 4	NO DATA	[33 32 25 32]	NO DATA
Bordetella				
pertussis	Tohama I	NO DATA	[26 33 39 24]	NO DATA
Burkholderia	1			1
cepacia	J2315	NO DATA	[25 37 33 27]	NO DATA
Burkholderia	1			
pseudomallei	K96243	NO DATA	[25 37 34 26]	NO DATA
Neisseria	1			
gonorrhoeae	FA 1090, ATCC 700825	[17 23 22 10]	[29 31 32 30]	NO DATA
Neisseria			l .	
meningitidis	MC58 (serogroup B)	NO DATA	[29 30 32 31]	NO DATA
Neisseria	l		1.	
meningitidis	serogroup C, FAM18	NO DATA	[29 30 32 31]	NO DATA

war and a second				
Neisseria meningitidis	Z2491 (serogroup A)	NO DATA	[29 30 32 31]	NO DATA
Chlamydophila				
pneumoniae	TW-183	NO DATA	NO DATA	NO DATA
Chlamydophila	AR39	NO DATA	NO DATA	NO DATA
pneumoniae Chlamydophila	AR39	NO DATA	NO DATA	NO DAIA
pneumoniae	CWL029	NO DATA	NO DATA	NO DATA
Chlamydophila				
pneumoniae	J138	NO DATA	NO DATA	NO DATA
Corynebacterium	NCTC13129	NO DATA	NO DATA	NO DATA
diphtheriae Mycobacterium	RCTC13129	NO DATA	NO DAIR	NO DATA
avium	k10	NO DATA	NO DATA	NO DATA
Mycobacterium				
avium	104	NO DATA	NO DATA	NO DATA
Mycobacterium		NO DATA	NO DATA	NO DATA
tuberculosis Mycobacterium	CSU#93	NO DATA	NO DATA	NO DALIA
tuberculosis	CDC 1551	NO DATA	NO DATA	NO DATA
Mycobacterium				
tuberculosis	H37Rv (lab strain)	NO DATA	NO DATA	NO DATA
Mycoplasma	N129	NO DATA	NO DATA	NO DATA
pneumoniae Staphylococcus	M129	NO DATA	NO DATA	NO DALK
aureus	MRSA252	[17 20 21 17]	[30 27 30 35]	[36 24 19 26]
Staphylococcus				
aureus	MSSA476	[17 20 21 17]	[30 27 30 35]	[36 24 19 26]
Staphylococcus		[17 20 21 17]	[30 27 30 35]	[35 24 19 27]
aureus Staphylococcus	COL	[17 20 21 17]	[30 27 30 35]	[35 24 19 27]
aureus	Mu50	[17 20 21 17]	[30 27 30 35]	[36 24 19 26]
Staphylococcus	11434			
aureus	MW2	[17 20 21 17]	[30 27 30 35]	[36 24 19 26]
Staphylococcus		f= 0 00 01 171	[30 27 30 35]	[36 24 19 26]
aureus Staphylococcus	N315	[17 20 21 17]	[30 27 30 33]	[36 24 19 20]
aureus	NCTC 8325	[17 20 21 17]	[30 27 30 35]	[35 24 19 27]
Streptococcus				
agalactiae	NEM316	[22 20 19 14]	[26 31 27 38]	[29 26 22 28]
Streptococcus		[22 21 19 13]	NO DATA	NO DATA
equi Streptococcus	NC_002955	[22 21 19 13]	NO DATA	NO DAIA
pyogenes	MGAS8232	[23 21 19 12]	[24 32 30 36]	NO DATA
Streptococcus				
pyogenes	MGAS315	[23 21 19 12]	[24 32 30 36]	NO DATA
Streptococcus	ssi-1	[23 21 19 12]	[24 32 30 36]	NO DATA
pyogenes Streptococcus	881-1	[23 21 19 12]	[24 32 30 30]	NO DAIA
pyogenes	MGAS10394	[23 21 19 12]	[24 32 30 36]	NO DATA
Streptococcus				
pyogenes	Manfredo (M5)	[23 21 19 12]	[24 32 30 36]	NO DATA
Streptococcus	SF370 (M1)	[23 21 19 12]	[24 32 30 36]	NO DATA
pyogenes Streptococcus	21310 (MI)	[23 61 13 12]	[22 32 30 30]	Pain
pneumoniae	670	[22 20 19 14]	[25 33 29 35]	[30 29 21 25]
Streptococcus			1.	
pneumoniae	R6	[22 20 19 14]	[25 33 29 35]	[30 29 21 25]
Streptococcus pneumoniae	TIGR4	[22 20 19 14]	[25 33 29 35]	[30 29 21 25]
pneumoniae Streptococcus	TIGHT	[106 40 17 14]	1 23 33 47 331	,-, =, == =3)
gordonii	NCTC7868	[21 21 19 14]	NO DATA	[29 26 22 28]
Streptococcus				
mitis	NCTC 12261	[22 20 19 14]	[26 30 32 34]	NO DATA
Streptococcus mutans	UA159	NO DATA	NO DATA	NO DATA
mutans	UA159	I NO DATA	I NO DATA	I wo min

Table 7D – Base Compositions of Common Respiratory Pathogens for Bioagent Identifying
Amplicons Corresponding to Primer Pair Nos: 355, 358, and 359

		Primer 355	Primer 358	Primer 359
Organism	Strain	[AGCT]	[AGCT]	[AGCT]
Klebsiella		1		
pneumoniae	MGH78578	NO DATA	[24 39 33 20]	[25 21 24 17]
	CO-92 Biovar			
Yersinia pestis	Orientalis	NO DATA	[26 34 35 21]	[23 23 19 22]
	KIMS P12 (Biovar			
Yersinia pestis	Mediaevalis)	NO DATA	[26 34 35 21]	[23 23 19 22]
Yersinia pestis	91001	NO DATA	[26 34 35 21]	[23 23 19 22]
Haemophilus			1	
influenzae	KW20	NO DATA	NO DATA	NO DATA
Pseudomonas				1
aeruginosa	PAO1	NO DATA	NO DATA	NO DATA
Pseudomonas				l
fluorescens	Pf0-1	NO DATA	NO DATA	NO DATA
Pseudomonas	1.			
putida	KT2440	NO DATA	[21 37 37 21]	NO DATA
Legionella		i .		NO DATA
pneumophila	Philadelphia-1	NO DATA	NO DATA	NO DATA
Francisella		l		NO DATA
tularensis	schu 4	NO DATA	NO DATA	NO DATA
Bordetella		l		pam
pertussis	Tohama I	NO DATA	NO DATA	NO DATA
Burkholderia		l		NO DAME
cepacia	J2315	NO DATA	NO DATA	NO DATA
Burkholderia			NO DATA	NO DATA
pseudomallei	K96243	NO DATA	NO DATA	NO DATA
Neisseria		NO DATA	NO DATA	NO DATA
gonorrhoeae	FA 1090, ATCC 700825	NO DATA	NO DATA	NO DATA
Neisseria meningitidis	Mass (n)	NO DATA	NO DATA	NO DATA
	MC58 (serogroup B)	NO DATA	NO DATA	NO DATA
Neisseria		NO DATA	NO DATA	NO DATA
meningitidis	serogroup C, FAN18	NO DATA	NO DATA	NO DATA
Neisseria meningitidis	Z2491 (serogroup A)	NO DATA	NO DATA	NO DATA
Chlamydophila	22491 (serogroup A)	NO DATA	NO DATA	NO DRIA
pneumoniae	TW-183	NO DATA	NO DATA	NO DATA
Chlamydophila	1W-103	NO DATA	NO BILLI	
pneumoniae	AR39	NO DATA	NO DATA	NO DATA
Chlamydophila	AK39	NO DALL	NO DILLI	
pneumoniae	CWL029	NO DATA	NO DATA	NO DATA
Chlamydophila	CHECKE			
pneumoniae	J138	NO DATA	NO DATA	NO DATA
Corynebacterium	10200			
diphtheriae	NCTC13129	NO DATA	NO DATA	NO DATA
Mycobacterium				
avium	k10	NO DATA	NO DATA	NO DATA
Mycobacterium		T	1	
avium	104	NO DATA	NO DATA	NO DATA
Mycobacterium		1	1	
tuberculosis	CSU#93	NO DATA	NO DATA	NO DATA
Mycobacterium				
tuberculosis	CDC 1551	NO DATA	NO DATA	NO DATA
Mycobacterium				
tuberculosis	H37Rv (lab strain)	NO DATA	NO DATA	NO DATA
Mycoplasma				
pneumoniae	M129	NO DATA	NO DATA	NO DATA
Staphylococcus				
aureus	MRSA252	NO DATA	NO DATA	NO DATA
Staphylococcus				
aureus	MSSA476	NO DATA	NO DATA	NO DATA
Staphylococcus				
aureus	COL	NO DATA	NO DATA	NO DATA
Staphylococcus				
aureus	Mu50	NO DATA	NO DATA	NO DATA

Staphylococcus aureus	MW2	NO DATA	NO DATA	NO DATA
Staphylococcus	PIWZ	NO DALA		
aureus	N315	NO DATA	NO DATA	NO DATA
Staphylococcus	1			
aureus	NCTC 8325	NO DATA	NO DATA	NO DATA
Streptococcus				
agalactiae	NEM316	NO DATA	NO DATA	NO DATA
Streptococcus				
equi	NC_002955	NO DATA	NO DATA	NO DATA
Streptococcus				1
pyogenes	MGAS8232	NO DATA	NO DATA	NO DATA
Streptococcus	1			l
pyogenes	MGAS315	NO DATA	NO DATA	NO DATA
Streptococcus	1	l		NO DATA
pyogenes	SSI-1	NO DATA	NO DATA	NO DATA
Streptococcus		100 Dams	NO DATA	NO DATA
pyogenes	NGAS10394	NO DATA	NO DATA	NO DATA
Streptococcus	Manfredo (M5)	NO DATA	NO DATA	NO DATA
pyogenes	MARITEGO (M5)	NO DATA	NO DATA	NO DATA
Streptococcus pyogenes	SF370 (M1)	NO DATA	NO DATA	NO DATA
Streptococcus	SE370 (ML)	INO DATA	NO DALK	
pneumoniae	670	NO DATA	NO DATA	NO DATA
Streptococcus	1			
pneumoniae	R6	NO DATA	NO DATA	NO DATA
Streptococcus				
pneumoniae	TIGR4	NO DATA	NO DATA	NO DATA
Streptococcus				
gordonii	NCTC7868	NO DATA	NO DATA	NO DATA
Streptococcus		1		1 .
mitis	NCTC 12261	NO DATA	NO DATA	NO DATA
Streptococcus	1	1 .		1
mutans	UA159	NO DATA	NO DATA	NO DATA

Table 7E – Base Compositions of Common Respiratory Pathogens for Bioagent Identifying
Amplicons Corresponding to Primer Pair Nos: 362, 363, and 367

Organism	Strain	Primer 362 [A G C T]	Primer 363 [A G C T]	Primer 367 [A G C T]
Klehsiella	Bulan	IN G G AI	11.000	
pneumoniae	MGH78578	[21 33 22 16]	[16 34 26 26]	NO DATA
phounomado	CO-92 Biovar	(00 00 00 00)	141 11 11	
Yersinia pestis	Orientalis	[20 34 18 20]	NO DATA	NO DATA
TORDINIA PODULE	KIMS Pl2 (Biovar	100 01 00 012		
Yersinia pestis	Mediaevalis)	[20 34 18 20]	NO DATA	NO DATA
Yersinia pestis	91001	[20 34 18 20]	NO DATA	NO DATA
Haemophilus				
influenzae	KW20	NO DATA	NO DATA	NO DATA
Pseudomonas				
aeruginosa	PAO1	[19 35 21 17]	[16 36 28 22]	NO DATA
Pseudomonas				
fluorescens	Pf0-1	NO DATA	[18 35 26 23]	NO DATA
Pseudomonas				
putida	KT2440	NO DATA	[16 35 28 23]	NO DATA
Legionella				
pneumophila	Philadelphia-1	NO DATA	NO DATA	NO DATA
Francisella				j .
tularensis	schu 4	NO DATA	NO DATA	NO DATA
Bordetella			l .	
pertussis	Tohama I	[20 31 24 17]	[15 34 32 21]	[26 25 34 19]
Burkholderia				
cepacia	J2315	[20 33 21 18]	[15 36 26 25]	[25 27 32 20]
Burkholderia			i	1
pseudomallei	K96243	[19 34 19 20]	[15 37 28 22]	[25 27 32 20]
Neisseria				
gonoxxhoeae	FA 1090, ATCC 700825	NO DATA	NO DATA	NO DATA

T 1		T		
Neisseria meningitidis	MC58 (serogroup B)	NO DATA	NO DATA	NO DATA
Neisseria				
meningitidis Neisseria	serogroup C, FAM18	NO DATA	NO DATA	NO DATA
meningitidis	Z2491 (serogroup A)	NO DATA	NO DATA	NO DATA
Chlamydophila				
pneumoniae	TW-183	NO DATA	NO DATA	NO DATA
Chlamydophila pneumoniae	AR39	NO DATA	NO DATA	NO DATA
Chlamydophila				
pneumoniae	CWL029	NO DATA	NO DATA	NO DATA
Chlamydophila pneumoniae	J138	NO DATA	NO DATA	NO DATA
Corynebacterium				
diphtheriae	NCTC13129	NO DATA	NO DATA	NO DATA
Mycobacterium avium	k10	[19 34 23 16]	NO DATA	[24 26 35 19]
Mycobacterium				
avium	104	[19 34 23 16]	NO DATA	[24 26 35 19]
Mycobacterium tuberculosis	CSU#93	[19 31 25 17]	NO DATA	[25 25 34 20]
Mycobacterium				
tuberculosis	CDC 1551	[19 31 24 18]	NO DATA	[25 25 34 20]
Mycobacterium tuberculosis	H37Rv (lab strain)	[19 31 24 18]	NO DATA	[25 25 34 20]
Mycoplasma				
pneumoniae	M129	NO DATA	NO DATA	NO DATA
Staphylococcus aureus	MRSA252	NO DATA	NO DATA	NO DATA
Staphylococcus				
aureus	MSSA476	NO DATA	NO DATA	NO DATA
Staphylococcus aureus	COL	NO DATA	NO DATA	NO DATA
Staphylococcus				
Staphylogoggus	Mu50	NO DATA	NO DATA	NO DATA
aureus	MW2	NO DATA	NO DATA	NO DATA
Staphylococcus				
Staphylococcus	N315	NO DATA	NO DATA	NO DATA
aureus	NCTC 8325	NO DATA	NO DATA	NO DATA
Streptococcus				
agalactiae Streptococcus	NEM316	NO DATA	NO DATA	NO DATA
equi	NC 002955	NO DATA	NO DATA	NO DATA
Streptococcus				
pyogenes Streptococcus	MGAS8232	NO DATA	NO DATA	NO DATA
pyogenes	MGAS315	NO DATA	NO DATA	NO DATA
Streptococcus				
pyogenes Streptococcus	SSI-1	NO DATA	NO DATA	NO DATA
pyogenes	MGAS10394	NO DATA	NO DATA	NO DATA
Streptococcus			n.m.	NO DATA
pyogenes Streptococcus	Manfredo (M5)	NO DATA	NO DATA	NO DATA
pyogenes	SF370 (M1)	NO DATA	NO DATA	NO DATA
Streptococcus			nama	n.m.
pneumoniae Streptococcus	670	NO DATA	NO DATA	NO DATA
pneumoniae	R6	[20 30 19 23]	NO DATA	NO DATA
Streptococcus		(an an an an)	NO DATA	NO DATA
pneumoniae Streptococcus	TIGR4	[20 30 19 23]	NO DATA	NO DATA
gordonii	NCTC7868	NO DATA	NO DATA	NO DATA
Streptococcus			NO DATA	NO DATA
mitis Streptococcus	NCTC 12261 UA159	NO DATA	NO DATA	NO DATA
berepeococcus	I OUTS	I no bitti	1	1

mutans

[396] Four sets of throat samples from military recruits at different military facilities taken at different time points were analyzed using the primers of the present invention. The first set was collected at a military training center from November 1 to December 20, 2002 during one of the most severe outbreaks of pneumonia associated with group A Streptococcus in the United States since 1968. During this outbreak, fifty-one throat swabs were taken from both healthy and hospitalized recruits and plated on blood agar for selection of putative group A Streptococcus colonies. A second set of 15 original patient specimens was taken during the height of this group A Streptococcus -associated respiratory disease outbreak. The third set were historical samples, including twenty-seven isolates of group A Streptococcus, from disease outbreaks at this and other military training facilities during previous years. The fourth set of samples was collected from five geographically separated military facilities in the continental U.S. in the winter immediately following the severe November/December 2002 outbreak.

[397] Pure colonies isolated from group A Streptococcus-selective media from all four collection periods were analyzed with the surveillance primer set. All samples showed base compositions that precisely matched the four completely sequenced strains of Streptococcus progenes. Shown in Figure 4 is a 3D diagram of base composition (axes A, G and C) of bioagent identifying amplicons obtained with primer pair number 14 (a precursor of primer pair number 348 which targets 165 rRNA). The diagram indicates that the experimentally determined base compositions of the clinical samples closely match the base compositions expected for Streptococcus progenes and are distinct from the expected base compositions of other organisms.

[398] In addition to the identification of Streptococcus pyogenes, other potentially pathogenic organisms were identified concurrently. Mass spectral analysis of a sample whose nucleic acid was amplified by primer pair number 349 (SEQ ID NOs: 401:1156) exhibited signals of bioagent identifying amplicons with molecular masses that were found to correspond to analogous base compositions of bioagent identifying amplicons of Streptococcus pyogenes (A27 G32 C24 T18), Neisseria meningitidis (A25 G27 C22 T18), and Haemophilus influenzae (A28 G28 C25 T20) (see Figure 5 and Table 7B). These organisms were present in a ratio of 4:5:20 as determined by comparison of peak heights with peak height of an internal PCR calibration standard as described in commonly owned U.S. Patent Application Serial No: 60/545,425 which is incorporated herein by reference in its entirety.

[399] Since certain division-wide primers that target housekeeping genes are designed to provide coverage of specific divisions of bacteria to increase the confidence level for identification of bacterial species, they are not expected to yield bioagent identifying amplicons for organisms outside of the specific divisions. For example, primer pair number 356 (SEQ ID NOs: 449:1380) primarily amplifies the nucleic acid of members of the classes Bacilli and Clostridia and is not expected to amplify proteobacteria such as Neisseria meningitidis and Haemophilus influenzae. As expected, analysis of the mass spectrum of amplification products obtained with primer pair number 356 does not indicate the presence of Neisseria meningitidis and Haemophilus influenzae but does indicate the presence of Streptococcus progenes (Figures 3 and 6, Table 7B). Thus, these primers or types of primers can confirm the absence of particular biospents from a sample.

[400] The 15 throat swabs from military recruits were found to contain a relatively small set of microbes in high abundance. The most common were Haemophilus influenza, Neisseria meningtitides, and Streptococcus pyogenes. Staphylococcus epidermidis, Moraxella cattarhalis, Corynebacterium pseudodiphtheriticum, and Staphylococcus aureus were present in fewer samples. An equal number of samples from healthy volunteers from three different geographic locations, were identically analyzed. Results indicated that the healthy volunteers have bacterial flora dominated by multiple, commensal non-beta-hemolytic Streptococcal species, including the viridans group streptococci (S. parasangunis, S. vestibularis, S. mitis, S. oralis and S. pneumoniae; data not shown), and none of the organisms found in the military recruits were found in the healthy controls at concentrations detectable by mass spectrometry. Thus, the military recruits in the midst of a respiratory disease outbreak had a dramatically different microbial population than that experienced by the general population in the absence of cridemic disease.

Example 7: Triangulation Genotyping Analysis for Determination of emm-Type of Streptococcus pyogenes in Epidemic Surveillance

[401] As a continuation of the epidemic surveillance investigation of Example 6, determination of sub-species characteristics (genotyping) of Streptococcus progenes, was carried out based on a strategy that generates strain-specific signatures according to the rationale of Multi-Locus Sequence Typing (MLST). In classic MLST analysis, internal fragments of several housekeeping genes are amplified and sequenced (Enright et al. Infection and Immunity, 2001, 69, 2416-2427). In classic MLST analysis, internal fragments of several housekeeping genes are amplified and sequenced. In the present investigation, bioagent identifying amplicons from housekeeping genes were produced using drill-down primers and analyzed by mass spectrometry. Since mass spectral analysis results in molecular mass.

from which base composition can be determined, the challenge was to determine whether resolution of emm classification of strains of Streptococcus pyogenes could be determined.

[402] For the purpose of development of a triangulation genotyping assay, an alignment was constructed of concatenated alleles of seven MLST housekeeping genes (glucose kinase (gki), glutamine transporter protein (gtr), glutamine transporter protein (gtr), glutamine transporter protein (gtr), glutamate racemase (murf), DNA mismatch repair protein (mutS), xanthine phosphoribosyl transferase (xpt), and acetyl-CoA acetyl transferase (xptL)) from cach of the 212 previously emm-typed strains of Streptococcus pyogenes. From this alignment, the number and location of primer pairs that would maximize strain identification via base composition was determined. As a result, 6 primer pairs were chosen as standard drill-down primers for determination of emm-type of Streptococcus pyogenes. These six primer pairs are displayed in Table 8. This drill-down set comprises primers with T modifications (note TMOD designation in primer names) which constitutes a functional improvement with regard to prevention of non-templated adenylation (vide supra) relative to originally selected primers which are displayed below in the same row.

Table 8: Triangulation Genotyping Analysis Primer Pairs for Group A Streptococcus Drill-Down

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
442	SP101_SPET11_358_387_ TMOD_F	588	SP101_SPET11_448_ 473_TMOD_R	998	gki
80	SP101_SPET11_358_387_	126	SP101_SPET11_448_ 473 TMOD_R	766	gki
443	SP101_SPET11_600_629_ TMOD_F	348	SP101_SPET11_686_ 714_TMOD_R	1018	gtr
81	SP101_SPET11_600_629_	62	SP101_SPET11_686_ 714 R	772	gtr
426	SP101_SPET11_1314_133 6_TMOD_F	363	SP101_SPET11_1403 _1431_TMOD_R	849	murI
86	SP101_SPET11_1314_133 6_F	68	SP101_SPET11_1403 _1431_R	711	muri
430	SP101_SPET11_1807_183 5_TMOD_F	235	SP101_SPET11_1901 _1927_TMOD_R	1439	mutS
90	SP101_SPET11_1807_183 5_F	33	SP101_SPET11_1901 _1927_R	1412	muts
438	SP101_SPET11_3075_310 3_TMOD_F	473	SP101_SPET11_3168 _3196_TMOD_R	875	xpt
96	SP101_SPET11_3075_310 3 F	108	SP101_SPET11_3168 3196 R	715	xpt
441	SP101_SPET11_3511_353 5_TMOD_F	531	SP101_SPET11_3605 _3629_TMOD_R	1294	yqiL
98	SP101_SPET11_3511_353 5 F	116	SP101_SPET11_3605 3629 R	832	yqiL

[403] The primers of Table 8 were used to produce bioagent identifying amplicons from nucleic acid present in the clinical samples. The bioagent identifying amplicons which were subsequently analyzed by mass spectrometry and base compositions corresponding to the molecular masses were calculated.

[404] Of the 51 samples taken during the peak of the November/December 2002 epidemic (Table 9A-C rows 1-3), all except three samples were found to represent *emm3*, a Group A *Streptococcus* genotype previously associated with high respiratory virulence. The three outliers were from samples obtained from healthy individuals and probably represent non-epidemic strains. Archived samples (Tables 9A-C rows 5-13) from historical collections showed a greater heterogeneity of base compositions and *emm* types as would be expected from different epidemics occurring at different places and dates. The results of the mass spectrometry analysis and *emm* gene sequencing were found to be concordant for the epidemic and historical samples.

Table 9A: Base Composition Analysis of Bioagent Identifying Amplicons of Group A

Streptococcus samples from Six Military Installations Obtained with Primer Pair Nos. 426 and 430

# of Instances	emm-type by Mass Spectrometry	emm-Gene Sequencing	Location (sample)	Year	murI (Primer Pair No. 426)	mutS (Primer Pair No. 430)
48	3	3	MCRD San	1	A39 G25 C20 T34	A38 G27 C23 T33
2	6	6	Diego	2002	A40 G24 C20 T34	A38 G27 C23 T33
1	28	28	(Cultured)	2002	A39 G25 C20 T34	A38 G27 C23 T33
15	3	ND	(Culturea)		A39 G25 C20 T34	A38 G27 C23 T33
6	3	3			A39 G25 C20 T34	A38 G27 C23 T33
3	5,58	5		l	A40 G24 C20 T34	A38 G27 C23 T33
6	6	6	NHRC San	l	A40 G24 C20 T34	A38 G27 C23 T33
1	11	11	Diego-	1	A39 G25 C20 T34	A38 G27 C23 T33
3	12	12	Archive	2003	A40 G24 C20 T34	A38 G26 C24 T33
1	22	22	(Cultured)		A39 G25 C20 T34	A38 G27 C23 T33
3	25,75	75	(Cultured)	l	A39 G25 C20 T34	A38 G27 C23 T33
4	44/61,82,9	44/61			A40 G24 C20 T34	A38 G26 C24 T33
2	53,91	91			A39 G25 C20 T34	A38 G27 C23 T33
1	2	2			A39 G25 C20 T34	A38 G27 C24 T32
2	3	3			A39 G25 C20 T34	A38 G27 C23 T33
1	4	4		1	A39 G25 C20 T34	A38 G27 C23 T33
1	6	6	Pt.	1	A40 G24 C20 T34	A38 G27 C23 T33
11	25 or 75	75	Leonard Wood	2003	A39 G25 C20 T34	A38 G27 C23 T33
1	25,75, 33, 34,4,52,84	75	(Cultured)		A39 G25 C20 T34	A38 G27 C23 T33
1	44/61 or 82 or 9	44/61			A40 G24 C20 T34	A38 G26 C24 T33
2	5 or 58	5	L		A40 G24 C20 T34	A38 G27 C23 T33
3	1	1			A40 G24 C20 T34	A38 G27 C23 T33
2	3	3	Ft. Sill	2003	A39 G25 C20 T34	A38 G27 C23 T33
1	4	4	(Cultured)	1 2005	A39 G25 C20 T34	A38 G27 C23 T33
1	28	28			A39 G25 C20 T34	A38 G27 C23 T33
1	3	3	Ft.	2003	A39 G25 C20 T34	A38 G27 C23 T33

1	4	4	Benning	1	A39 G25 C20 T34	A38 G27 C23 T33
3	6	6	(Cultured)		A40 G24 C20 T34	A38 G27 C23 T33
1	11	11] '		A39 G25 C20 T34	A38 G27 C23 T33
1	13	94**			A40 G24 C20 T34	A38 G27 C23 T33
1	44/61 or 82 or 9	82			A40 G24 C20 T34	A38 G26 C24 T33
1	5 or 58	58		l	A40 G24 C20 T34	A38 G27 C23 T33
1	78 or 89	89	}		A39 G25 C20 T34	A38 G27 C23 T33
2	5 or 58		Lackland		A40 G24 C20 T34	A38 G27 C23 T33
1	2		AFB	ĺ	A39 G25 C20 T34	A38 G27 C24 T32
1	81 or 90	ND		2003	A40 G24 C20 T34	A38 G27 C23 T33
1	78	l	(Throat Swabs)		A38 G26 C20 T34	A38 G27 C23 T33
3***	No detection		Swaus,		No detection	No detection
7	3	ND			A39 G25 C20 T34	A38 G27 C23 T33
1	3	ND	MCRD San	l	No detection	A38 G27 C23 T33
1	3	ND	Diego	2002	No detection	No detection
1	3	ND	(Throat	2002	No detection	No detection
2	3	ND	Swabs)	4	No detection	A38 G27 C23 T33
3	No detection	ND	1		No detection	No detection

Table 9B: Base Composition Analysis of Bioagent Identifying Amplicons of Group A

Streptococcus samples from Six Military Installations Obtained with Primer Pair Nos. 438 and 441

# of Instances	emm-type by Mass Spectrometry	emm-Gene Sequencing	Location (sample)	Year	xpt (Primer Pair No. 438)	yqiL (Primer Pair No. 441)
48	3	3	MCRD San		A30 G36 C20 T36	A40 G29 C19 T31
2	6	6	Diego		A30 G36 C20 T36	A40 G29 C19 T31
1	28	28	(Cultured)	2002	A30 G36 C20 T36	A41 G28 C18 T32
15	3	ND	(Curtured)		A30 G36 C20 T36	A40 G29 C19 T31
6	3	3			A30 G36 C20 T36	A40 G29 C19 T31
3	5,58	5			A30 G36 C20 T36	A40 G29 C19 T31
6	6	6	NHRC San		A30 G36 C20 T36	A40 G29 C19 T31
1	11	11	Diego-		A30 G36 C20 T36	A40 G29 C19 T31
3	12	12	Archive	(Cultured)	A30 G36 C19 T37	A40 G29 C19 T31
1	22	22	(Cultured)		A30 G36 C20 T36	A40 G29 C19 T31
3	25,75	75	(curcureu)		A30 G36 C20 T36	A40 G29 C19 T31
4	44/61,82,9	44/61			A30 G36 C20 T36	A41 G28 C19 T31
2	53,91	91			A30 G36 C19 T37	A40 G29 C19 T31
1	2	2			A30 G36 C20 T36	A40 G29 C19 T31
2	3	3			A30 G36 C20 T36	A40 G29 C19 T31
1	4	4			A30 G36 C19 T37	A41 G28 C19 T31
1	6	6	Ft. Leonard		A30 G36 C20 T36	A40 G29 C19 T31
11	25 or 75	75	Mood	2003	A30 G36 C20 T36	A40 G29 C19 T31
1	25,75, 33, 34,4,52,84	75	(Cultured)		A30 G36 C19 T37	A40 G29 C19 T31
1	44/61 or 82 or 9	44/61	12.5		A30 G36 C20 T36	A41 G28 C19 T31
2	5 or 58	5	l		A30 G36 C20 T36	A40 G29 C19 T31
3	1	1	Ft. Sill	2003	A30 G36 C19 T37	A40 G29 C19 T31
2	3	3	(Cultured)		A30 G36 C20 T36	A40 G29 C19 T31
1	4	4	(ourcusou)		A30 G36 C19 T37	A41 G28 C19 T31

1	28	28			A30 G36 C20 T36	A41 G28 C18 T32
1	3	3		ĺ	A30 G36 C20 T36	A40 G29 C19 T31
1	4	4	1		A30 G36 C19 T37	A41 G28 C19 T31
3	6	6]	1	A30 G36 C20 T36	A40 G29 C19 T31
1.	11	11	Pt.		A30 G36 C20 T36	A40 G29 C19 T31
1	13	94**	Benning	2003	A30 G36 C20 T36	A41 G28 C19 T31
	44/61 or 82		(Cultured)			
1	or 9	82			A30 G36 C20 T36	A41 G28 C19 T31
1	5 or 58	58			A30 G36 C20 T36	A40 G29 C19 T31
1	78 or 89	89			A30 G36 C20 T36	A41 G28 C19 T31
2	5 or 58		Tunkland	Lackland AFR	A30 G36 C20 T36	A40 G29 C19 T31
1	2	l			A30 G36 C20 T36	A40 G29 C19 T31
1	81 or 90	ND		2003	A30 G36 C20 T36	A40 G29 C19 T31
1	78	1	(Throat Swabs)	A30 G36 C20 T36	A41 G28 C19 T31	
3***	No detection	1	Swaus)		No detection	No detection
7	3	ND			A30 G36 C20 T36	A40 G29 C19 T31
1	3	ND	MCRD San		A30 G36 C20 T36	A40 G29 C19 T31
1	3	ND	Diego	2002	A30 G36 C20 T36	No detection
1	3	ND	(Throat	2002	No detection	A40 G29 C19 T31
2	3	ND	Swabs)		A30 G36 C20 T36	A40 G29 C19 T31
3	No detection	ND	1	l	No detection	No detection

Table 9C: Base Composition Analysis of Bioagent Identifying Amplicons of Group A Streptococcus samples from Six Military Installations Obtained with Primer Pair Nos. 438 and 441

# of Instances	emm-type by Mass Spectrometry	emm-Gene Sequencing	Location (sample)	Year	gki (Primer Pair No. 442)	gtr ((Primer Pair No. 443)
48	3	3	MCRD San		A32 G35 C17 T32	A39 G28 C16 T32
2	6	6	Diego	2002	A31 G35 C17 T33	A39 G28 C15 T33
1	28	28	(Cultured)	2002	A30 G36 C17 T33	A39 G28 C16 T32
15	3	ND	(Cultureu)		A32 G35 C17 T32	A39 G28 C16 T32
6	3	3			A32 G35 C17 T32	A39 G28 C16 T32
3	5,58	5		1	A30 G36 C20 T30	A39 G28 C15 T33
6	6	6	NHRC San		A31 G35 C17 T33	A39 G28 C15 T33
1	11	11	Diego-		A30 G36 C20 T30	A39 G28 C16 T32
3	12	12	Archive	2003	A31 G35 C17 T33	A39 G28 C15 T33
1	22	22	(Cultured)		A31 G35 C17 T33	A38 G29 C15 T33
3	25,75	75] (Curcurea)	(Curcureu)	A30 G36 C17 T33	A39 G28 C15 T33
4	44/61,82,9	44/61		ŀ	A30 G36 C18 T32	A39 G28 C15 T33
2	53,91	91]	!	A32 G35 C17 T32	A39 G28 C16 T32
1	2	2			A30 G36 C17 T33	A39 G28 C15 T33
2	3	3			A32 G35 C17 T32	A39 G28 C16 T32
1	4	4			A31 G35 C17 T33	A39 G28 C15 T33
1	6	6	Ft.	1	A31 G35 C17 T33	A39 G28 C15 T33
11	25 or 75	75	Leonard Wood	2003	A30 G36 C17 T33	A39 G28 C15 T33
1	25,75, 33, 34,4,52,84	75	(Cultured)		A30 G36 C17 T33	A39 G28 C15 T33
1	44/61 or 82 or 9	44/61			A30 G36 C18 T32	A39 G28 C15 T33
2	5 or 58	5	l	<u> </u>	A30 G36 C20 T30	A39 G28 C15 T33
3	1	1	Ft. Sill	2003	A30 G36 C18 T32	A39 G28 C15 T33
2	3	3	1	1	A32 G35 C17 T32	A39 G28 C16 T32

1	4	4	(Cultured)	1	A31 G35 C17 T33	A39 G28 C15 T33
1	28	28			A30 G36 C17 T33	A39 G28 C16 T32
1	3	3]	l l	A32 G35 C17 T32	A39 G28 C16 T32
1	4	4			A31 G35 C17 T33	A39 G28 C15 T33
3	6	6		1	A31 G35 C17 T33	A39 G28 C15 T33
1	11	11	Pt. Benning		A30 G36 C20 T30	A39 G28 C16 T32
1	13	94**	Benning	2003	A30 G36 C19 T31	A39 G28 C15 T33
	44/61 or 82		(Cultured)			
1	or 9	82		1	A30 G36 C18 T32	A39 G28 C15 T33
1	5 or 58	58]	l	A30 G36 C20 T30	A39 G28 C15 T33
1	78 or 89	89			A30 G36 C18 T32	A39 G28 C15 T33
2	5 or 58		Lackland	ļ	A30 G36 C20 T30	A39 G28 C15 T33
1	2	i	AFB	l	A30 G36 C17 T33	A39 G28 C15 T33
1	81 or 90	ND		2003	A30 G36 C17 T33	A39 G28 C15 T33
1	78		(Throat Swabs)		A30 G36 C18 T32	A39 G28 C15 T33
3***	No detection		Owens,	l	No detection	No detection
7	3	ND			A32 G35 C17 T32	A39 G28 C16 T32
1	3	ND	MCRD San	i	No detection	No detection
1	3	ND	Diego	2002	A32 G35 C17 T32	A39 G28 C16 T32
1.	3	ND	(Throat	2002	A32 G35 C17 T32	No detection
2	3	ND	Swabs)	l	A32 G35 C17 T32	No detection
3	No detection	ND	1	l	No detection	No detection

Example 8: Design of Calibrant Polynucleotides based on Bioagent Identifying Amplicons for Identification of Species of Bacteria (Bacterial Bioagent Identifying Amplicons)

[405] This example describes the design of 19 calibrant polynucleotides based on bacterial bioagent identifying amplicons corresponding to the primers of the broad surveillance set (Table 5) and the Bacillus anthracis drill-down set (Table 6).

[406] Calibration sequences were designed to simulate bacterial bioagent identifying amplicons produced by the T modified primer pairs shown in Tables 5 and 6 (primer names have the designation "TMOD"). The calibration sequences were chosen as a representative member of the section of bacterial genome from specific bacterial species which would be amplified by a given primer pair. The model bacterial species upon which the calibration sequences are based are also shown in Table 10. For example, the calibration sequence chosen to correspond to an amplicon produced by primer pair no. 361 is SEQ ID NO: 1445. In Table 10, the forward (_F) or reverse (_R) primer name indicates the coordinates of an extraction representing a gene of a standard reference bacterial genome to which the primer hybridizes e.g.: the forward primer name 168_EC_713_732_TMOD_F indicates that the forward primer hybridizes to residues 713-732 of the gene encoding 168 ribosomal RNA in an E. coli reference sequence (in this case, the reference sequence is an extraction consisting of residues 4033120-4034661 of the genomic sequence of E. coli K12 (GenBank gi number 16127994). Additional gene coordinate reference information is shown in Table 11. The designation "TMOD" in the primer names indicates that the 5' end of the primer has been modified with a non-matched template T residue which

prevents the PCR polymerase from adding non-templated adenosine residues to the 5' end of the amplification product, an occurrence which may result in miscalculation of base composition from molecular mass data (vide supra).

[0143] The 19 calibration sequences described in Tables 10 and 11 were combined into a single calibration polynucleotide sequence (EEQ ID NO: 1464 - which is herein designated a "combination calibration polynucleotide") which was then cloned into a pCR®-Blunt vector (Invitrogen, Carlsbad, CA). This combination calibration polynucleotide can be used in conjunction with the primers of Tables 5 or 6 as an internal standard to produce calibration amplicons for use in determination of the quantity of any bacterial bioagent. Thus, for example, when the combination calibration polynucleotide vector is present in an amplification reaction mixture, a calibration amplicon based on primer pair 346 (16S rRNA) will be produced in an amplification reaction with primer pair 346 and a calibration amplicon based on primer pair 363 (rpoC) will be produced with primer pair 363. Coordinates of each of the 19 calibration sequences within the calibration polynucleotide (SEQ ID NO: 1464) are indicated in Table

Table 10: Bacterial Primer Pairs for Production of Bacterial Bioagent Identifying
Amplicons and Corresponding Representative Calibration Sequences

Primer Paîr No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Calibration Sequence Model Species	Calibration Sequence (SEQ ID NO:)
361	16S_EC_1090_1111_2_T MOD_F	697	168_EC_1175_1196_TMOD_R	1398	Bacillus anthracis	1445
346	168_EC_713_732_TMOD_ F	202	16S_EC_789_809_TMOD_R	1110	Bacillus anthracis	1446
347	168_EC_785_806_TMOD_ F	560	168_EC_880_897_TMOD_R	1278	Bacillus anthracis	1447
348	168_EC_960_981_TMOD_ F	706	16S_EC_1054_1073_TMOD_R	895	Bacillus anthracis	1448
349	23S_EC_1826_1843_TMO D_F	401	23S_EC_1906_1924_TMOD_R	1156	Bacillus anthracis	1449
360	238_EC_2646_2667_TMO D_F	409	238_EC_2745_2765_TMOD_R	1434	Bacillus anthracis	1450
350	CAPC_BA_274_303_TMOD _F	476	CAPC_BA_349_376_TMOD_R	1314	Bacillus anthracis	1451
351	CYA_BA_1353_1379_TMO D_F	355	CYA_BA_1448_1467_TMOD_R	1423	Bacillus anthracis	1452
352	INFB_BC_1365_1393_TM OD_F	687	INFB_EC_1439_1467_TMOD_ R	1411	Bacillus anthracis	1453
353	LEF_BA_756_781_TMOD_ F	220	LEF_BA_843_872_TMOD_R	1394	Bacillus anthracis	1454
356	RPLB_EC_650_679_TMOD F	449	RPLB_BC_739_762_TMOD_R	1380	Clostridium botulinum	1455
449	RPLB_EC_690_710_F	309	RPLB_EC_737_758_R	1336	Clostridium botulinum	1456
359	RPOB_EC_1845_1866_TM OD_F	659	RPOB_EC_1909_1929_TMOD_ R	1250	Yersinia Pestis	1457
362	RPOB_EC_3799_3821_TM OD_F	581	RPOB_EC_3862_3888_TMOD_ R	1325	Burkholderia mallei	1458
363	RPOC_EC_2146_2174_TM OD_F	284	RPOC_EC_2227_2245_TMOD_ R	898	Burkholderia mallei	1459

354	RPOC_EC_2218_2241_TM OD_F	405	RPOC_EC_2313_2337_TMOD_	1072	Bacillus anthracis	1460
355	5SPE_BA_115_137_TMOD F	255	SSPE_BA_197_222_TMOD_R	1402	Bacillus anthracis	1461
367	TUFB_EC_957_979_TMOD	308	TUFB_EC_1034_1058_TMOD_ R	1276	Burkholderia mallei	1462
358	VAL5_EC_1105_1124_TM	385	VALS_EC_1195_1218_TMOD_ R	1093	Yersinia Pestis	1463

Table 11: Primer Pair Gene Coordinate References and Calibration Polynucleotide Sequence
Coordinates within the Combination Calibration Polynucleotide

				a Markey of
Bactcrial	Gone Extraction	Reference GenBank GI	Primer	Coordinates of
Gene and	Coordinates	No. of Genomic (G) or	Pair No.	Calibration Sequence in
Species	of Genemic or Plasmid	Plasmid (P) Sequence		Combination Calibration
-	Sequence	l .		Polynucleotide (SEQ ID
				NO: 1464)
16S E. coli	40331204034661	16127994 (G)	346	16109
16S E. coli	40331204034661	16127994 (G)	347	83190
16S E. coli	40331204034661	16127994 (G)	348	246353
16S E. coli	40331204034661	16127994 (G)	361	368469
23S E. coli	41662204169123	16127994 (G)	349	743837
23S E. coli	41662204169123	16127994 (G)	360	865981
rpcB E.	41788234182851	16127994 (G)	359	15911672
coli.	(complement strand)			
rpoB E. coli	41788234182851	16127994 (G)	362	20812167
2000 01 0444	(complement strand)			
rpoC E. colí	41829284187151	16127994 (G)	354	18101926
rpoC E. coli	41829284187151	16127994 (G)	363	21832279
infB E. coli	33136553310983	16127994 (G)	352	16921791
Inib D. Coll	(complement strand)			
tufB E. coli	41735234174707	16127994 (G)	367	24002498
rplB E. coli	34490013448180	16127994 (G)	356	19452060
rplB E. coli	34490013448180	16127994 (G)	449	19862055
val5 E. coli	44814054478550	16127994 (G)	358	14621572
VALUE A. COLL	(complement strand)			
capC	5607455628 (complement	6470151 (P)	350	25172616
B. anthracis	strand)			
Cya	156626154288	4894216 (P)	351	13381449
B. anthracis	(complement strand)		1	
lef	127442129921	4894216 (P)	353	11211234
8. anthracis	20.440.1203500	1		1
sspE	226496226783	30253828 (G)	355	1007-1104
B. anthracis	220450220705			

Example 9: Use of a Calibration Polynucleotide for Determining the Quantity of *Bacillus*Anthracis in a Sample Containing a Mixture of Microbes

[407] The process described in this example is shown in Figure 2. The capC gene is a gene involved in capsule synthesis which resides on the pX02 plasmid of Bacillus anthracis. Primer pair number 350 (see Tables 10 and 11) was designed to identify Bacillus anthracis via production of a bacterial bioagent identifying amplicon. Known quantities of the combination calibration polynucleotide vector described in Example 8 were added to amplification mixtures containing bacterial bioagent nucleic acid from a mixture of microbes which included the Ames strain of Bacillus anthracis. Upon amplification of the bacterial bioagent nucleic acid and the combination calibration polynucleotide vector with primer pair no. 350, bacterial bioagent identifying amplicons and calibration amplicons were obtained and characterized by mass spectrometry. A mass spectrum measured for the amplification reaction is shown in Figure 7. The molecular masses of the bioagent identifying amplicons provided the means for identification of the bioagent from which they were obtained (Ames strain of Bacillus

authracis) and the molecular masses of the calibration amplicons provided the means for their identification as well. The relationship between the abundance (peak height) of the calibration amplicon signals and the bacterial bioagent identifying amplicon signals provides the means of calculation of the copies of the pXO2 plasmid of the Ames strain of Bacillus authracis. Methods of calculating quantities of molecules based on internal calibration procedures are well known to those of ordinary skill in the art.

[408] Averaging the results of 10 repetitions of the experiment described above, enabled a calculation that indicated that the quantity of Ames strain of *Bacillus anthracis* present in the sample corresponds to approximately 10 copies of pX02 plasmid.

Example 10: Triangulation Genotyping Analysis of Campylobacter Species

[409] A series of triangulation genotyping analysis primers were designed as described in Example 1 with the objective of identification of different strains of Campylobacter jejuni. The primers are listed in Table 12 with the designation "CIST_CJ." Housekeeping genes to which the primers hybridize and produce bioagent identifying amplicons include: tkt (transketolase), glyA (serine hydroxymethyltransferase), gltA (citrate synthase), aspA (aspartate ammonia lyase), glnA (glutamine synthase), pgm (phosphoglycerate mutase), and uncA (ATP synthetase alpha chain).

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
1053	CJST CJ 1080 1110 F	681	CJST_CJ_1166_1198_R	1022	gltA
1047	CJST CJ 584 616 F	315	CJST_CJ_663_692_R	1379	glnA
1048	CJST_CJ_360_394_F	346	CJST CJ 442 476 R	955	aspA
1049	CJST CJ 2636 2668 F	504	CJST_CJ_2753_2777_R	1409	tkt
1054	CJST CJ 2060 2090 F	323	CJST CJ 2148 2174 R	1068	pgm
1064	GTOT GT 1600 1712 P	479	CTOT OT 1705 1922 D	029	CT11/A

Table 12: Campylobacter Genotyping Primer Pairs

[410] The primers were used to amplify nucleic acid from 50 food product samples provided by the USDA, 25 of which contained Campylobacter jejuni and 25 of which contained Campylobacter coil. Primers used in this study were developed primarily for the discrimination of Campylobacter jejuni clonal complexes and for distinguishing Campylobacter jejuni from Campylobacter coil. Finer discrimination between Campylobacter coil types is also possible by using specific primers targeted to loci where closely-related Campylobacter coil isolates demonstrate polymorphisms between strains. The conclusions of the comparison of base composition analysis with sequence analysis are shown in Tables 13A-C.

Table 13A – Results of Base Composition Analysis of 50 Campylobacter Samples with Drill-down

MLST Primer Pair Nos: 1048 and 1047

Group	Species	Isolate origin	MLST type or Clonal Complex by Base Composition analysis	MUST Type or Clonal Complex by Sequence analysis	Strain	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1048 (aspA)	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1047 (glnA)
J-1	C. jejuni	Goose	ST 690 /692/707/991	ST 991	RM3673	A30 G25 C16 T46	A47 G21 C16 T25
J-2	C. jejuni	Human	Complex 206/48/353	ST 356, complex 353	RM4192	A30 G25 C16 T46	A48 G21 C17 T23
J-3	C. jejuni	Human	Complex 354/179	ST 436	RM4194	A30 G25 C15 T47	A48 G21 C18 T22
J-4	C. jejuni	Human	Complex 257	ST 257, complex 257	RM4197	A30 G25 C16 T46	A48 G21 C18 T22
J-5	C. ieiuni	Human	Complex 52	ST 52, complex 52	RM4277	A30 G25 C16 T46	A48 G21 C17 T23
J-6	C. jejuni	Human	Complex 443	ST 51, complex 443	RM4275 RM4279	A30 G25 C15 T47	A48 G21 C17 T23 A48 G21 C17 T23
J-7	c. jejuni	Human	Complex 42	ST 604, complex 42	RM1864	A30 G25 C15 T47	A48 G21 C18 T22
J-8	C. jejuni	Human	Complex 42/49/362	ST 362, complex 362	RM3193	A30 G25 C15 T47	A48 G21 C18 T22
J-9	C. jejuni	Human	Complex 45/283	ST 147, Complex 45	RM3203	A30 G25 Cl5 T47	A47 G21 C18 T23
	C. jejuni			ST 828	RM4183	A31 G27 C20 T39	A48 G21 C16 T24
		Human		ST 832	RM1169	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1056	RM1857	A31 G27 C20 T39	A48 G21 C16 T24
				ST 889	RM1166	A31 G27 C20 T39	A48 G21 C16 T24
				ST 829	RM1182	A31 G27 C20 T39	A48 G21 C16 T24
			l	ST 1050	RM1518	A31 G27 C20 T39	A48 G21 C16 T24
	1			ST 1051	RM1521	A31 G27 C20 T39	A48 G21 C16 T24
			Consistent	ST 1053	RM1523	A31 G27 C20 T39	A48 G21 C16 T24
		Poultry	with 74 closely	ST 1055	RM1527	A31 G27 C20 T39	A48 G21 C16 T24
	1		related sequence	ST 1017	RM1529	A31 G27 C20 T39	A48 G21 C16 T24
C-1	C. coli		types (none belong to a	ST 860	RM1840	A31 G27 C20 T39	A48 G21 C16 T24
		\	clonal complex)	ST 1063	RM2219	A31 G27 C20 T39	A48 G21 C16 T24
			1	ST 1066	RM2241	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1067	RM2243	A31 G27 C20 T39	A48 G21 C16 T24
	-]	ST 1068	RM2439	A31 G27 C20 T39	A48 G21 C16 T24
			1	ST 1016	RM3230	A31 G27 C20 T39	A48 G21 C16 T24
		Swine		ST 1069	RM3231	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1061	RM1904	A31 G27 C20 T39	A48 G21 C16 T24
	l	Unknown	1	ST 825	RM1534	A31 G27 C20 T39	A48 G21 C16 T24
				ST 901	RM1505	A31 G27 C20 T39	A48 G21 C16 T24
C-2	C. coli	Human	ST 895	ST 895	RM1532	A31 G27 C19 T40	A48 G21 C16 T24
C-3	C. coli	Poultry	Consistent	ST 1064	RM2223	A31 G27 C20 T39	A48 G21 C16 T24

	with 63 closely	ST 1082	RM1178	A31 G27 C20 T39	A48 G21 C16 T24
	related sequence	ST 1054	RM1525	A31 G27 C20 T39	A48 G21 C16 T24
	types (none belong to a	ST 1049	RM1517	A31 G27 C20 T39	A48 G21 C16 T24
Marmos	-11	ST 891	RM1531	A31 G27 C20 T39	A48 G21 C16 T24

Table 13B – Results of Base Composition Analysis of 50 *Campylobacter* Samples with

Drill-down MLST Primer Pair Nos: 1053 and 1064

Group	Species	Isolate origin	MLST type or Clonal Complex by Base Composition analysis	MLST Type or Clonal Complex by Soquence analysis	Strain	Base Composition of Bicagent Identifying Amplicon Obtained with Primor Pair No: 1053 (gltA)	Base Composition of Bloagent Identifying Amplicon Obtained with Primer Pair No: 1064 (glyA)
J-1	C. jejuni	Goose	ST 690 /692/707/991	ST 991	RM3673	A24 G25 C23 T47	A40 G29 C29 T45
J-2	c. jejuni	Human	Complex 206/48/353	ST 356, complex 353	RM4192	A24 G25 C23 T47	A40 G29 C29 T45
J-3	C. jejuni	Human	Complex 354/179	ST 436	RM4194	A24 G25 C23 T47	A40 G29 C29 T45
J-4	C. jejuni	Human	Complex 257	ST 257, complex 257	RM4197	A24 G25 C23 T47	A40 G29 C29 T45
J-5	C. jejuni	Human	Complex 52	ST 52, complex 52	RM4277	A24 G25 C23 T47	A39 G30 C26 T48
J-6	C.			ST 51,	RM4275	A24 G25 C23 T47	A39 G30 C28 T46
J-6	jejuni	Human	Complex 443	complex 443	RM4279	A24 G25 C23 T47	A39 G30 C28 T46
J-7	C. jejuni	Human	Complex 42	ST 604, complex 42	RM1864	A24 G25 C23 T47	A39 G30 C26 T48
J-8	C. jejuni	Human	Complex 42/49/362	ST 362, complex 362	RM3193	A24 G25 C23 T47	A38 G31 C28 T46
J-9	C. jejuni	Human	Complex 45/283	ST 147, Complex 45	RM3203	A24 G25 C23 T47	A38 G31 C28 T46
	C. jejuni		Consistent with 74	ST 828	RN4183	A23 G24 C26 T46	A39 G30 C27 T47
C-1	C. coli	Human	closely related	ST 832	RM1169	A23 G24 C26 T46	A39 G30 C27 T47
			sequence types (none	ST 1056	RM1857	A23 G24 C26 T46	A39 G30 C27 T47
			belong to a clonel	ST 889	RM1166	A23 G24 C26 T46	A39 G30 C27 T47
			complex)	ST 829	RM1182	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1050	RM1518	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1051	RM1521	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1053	RM1523	A23 G24 C26 T46	A39 G30 C27 T47
		Poultry		ST 1055	RM1527	A23 G24 C26 T46	A39 G30 C27 T47
		Foundly		ST 1017	RM1529	A23 G24 C26 T46	A39 G30 C27 T47
				ST 860	RM1840	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1063	RM2219	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1066	RM2241	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1067	RM2243	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1068	RM2439	A23 G24 C26 T46	A39 G30 C27 T47
		Swine		ST 1016	RM3230	A23 G24 C26 T46	A39 G30 C27 T47

1	1	I		ST 1069	RM3231	A23 G24 C26 T46	NO DATA
				ST 1061	RM1904	A23 G24 C26 T46	A39 G30 C27 T47
				ST 825	RM1534	A23 G24 C26 T46	A39 G30 C27 T47
		Unknown		ST 901	RM1505	A23 G24 C26 T46	A39 G30 C27 T47
C-2	C. coli	Human	ST 895	ST 895	RM1532	A23 G24 C26 T46	A39 G30 C27 T47
			Consistent with 63	ST 1064	RM2223	A23 G24 C26 T46	A39 G30 C27 T47
		Poultry	closely	ST 1082	RM1178	A23 G24 C26 T46	A39 G30 C27 T47
C-3	C. coli seque	sequence types (none	ST 1054	RM1525	A23 G24 C25 T47	A39 G30 C27 T47	
		belong to a	ST 1049	RM1517	A23 G24 C25 T46	A39 G30 C27 T47	
		Maxmoset	complex)	ST 891	RM1531	A23 G24 C26 T46	A39 G30 C27 T47

Table 13C – Results of Base Composition Analysis of 50 *Campylobacter* Samples with Drill-down MLST Primer Pair Nos: 1054 and 1049

Group	Species	Isolate origin	MLST type or Clonal Complex by Base Composition analysis	MLST Type or Clonal Complex by Sequence analysis	Strain	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1054 (pgm)	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1049 (tkt)
J-1	C. jejuni	Goose	ST 690 /692/707/991	ST 991	RM3673	A26 G33 C18 T38	A41 G28 C35 T38
J-2	c. jejuni	Human	Complex 206/48/353	ST 356, complex 353	RM4192	A26 G33 Cl9 T37	A41 G28 C36 T37
J-3	C. jejuni	Huma <i>rı</i>	Complex 354/179	ST 436	RM4194	A27 G32 C19 T37	A42 G28 C36 T36
J-4	C. jejuni	Human	Complex 257	ST 257, complex 257	RM4197	A27 G32 C19 T37	A41 G29 C35 T37
J-5	C. jejuni	Human	Complex 52	ST 52, complex 52	RM4277	A26 G33 C18 T38	A41 G28 C36 T37
7-6	c.	Human	Complex 443	ST 51, complex	RM4275	A27 G31 C19 T38	A41 G28 C36 T37
U-0	jejuni	nullan	Complex 443	443	RM4279	A27 G31 C19 T38	A41 G28 C36 T37
J-7	C. jejuni	Huma.n	Complex 42	ST 604, complex 42	RM1864	A27 G32 C19 T37	A42 G28 C35 T37
J-8	C. jejuni	Human	Complex 42/49/352	ST 362, complex 362	RM3193	A26 G33 C19 T37	A42 G28 C35 T37
J-9	C. jejuni	Human	Complex 45/283	ST 147, Complex 45	RM3203	A28 G31 C19 T37	A43 G28 C36 T35
	C. jejuni		Consistent with 74	ST 828	RM4183	A27 G30 C19 T39	A46 G28 C32 T36
C-1	C. coli	Human.	closely related	ST 832	RM1169	A27 G30 C19 T39	A46 G28 C32 T36
			types (none	ST 1056	RM1857	A27 G30 C19 T39	A46 G28 C32 T36
		Poultry	belong to a clonal	ST 889	RM1166	A27 G30 C19 T39	A46 G28 C32 T36
		-	complex)	ST 829	RM1182	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1050	RM1518	A27 G30 C19 T39	A46 G28 C32 T36
		1	1	ST 1051	RM1521	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1053	RM1523	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1055	RM1527	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1017	RM1529	A27 G30 C19 T39	A46 G28 C32 T36

	ì	1		ST 860	RM1840	A27 G30 C19 T39	A46 G28 C32 T36
			,	ST 1063	RM2219	A27 G30 C19 T39	A46 G28 C32 T36
	1			ST 1066	RM2241	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1067	RM2243	A27 G30 C19 T39	A46 G28 C32 T36
	}			ST 1068	RM2439	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1016	RM3230	A27 G30 C19 T39	A46 G28 C32 T36
		Swine		ST 1069	RM3231	A27 G30 C19 T39	A46 G28 C32 T36
	1			ST 1061	RM1904	A27 G30 C19 T39	A46 G28 C32 T36
	1			ST 825	RM1534	A27 G30 C19 T39	A46 G28 C32 T36
		Unknown		ST 901	RM1505	A27 G30 C19 T39	A46 G28 C32 T36
C=2	C. coli	Human	ST 895	ST 895	RM1532	A27 G30 C19 T39	A45 G29 C32 T36
			Consistent	ST 1064	RM2223	A27 G30 C19 T39	A45 G29 C32 T36
			with 63 closely	ST 1082	RN1178	A27 G30 C19 T39	A45 G29 C32 T36
C-3	C. coli	Poultry	related sequence	ST 1054	RM1525	A27 G30 C19 T39	A45 G29 C32 T36
			types (none belong to a	ST 1049	RM1517	A27 G30 C19 T39	A45 G29 C32 T36
		Marmoset	clonal complex)	ST 891	RM1531	A27 G30 C19 T39	A45 G29 C32 T36

[411] The base composition analysis method was successful in identification of 12 different strain groups. Campylobacter jejuni and Campylobacter coli are generally differentiated by all loci. Ten clearly differentiated Campylobacter jejuni isolates and 2 major Campylobacter coli groups were identified even though the primers were designed for strain typing of Campylobacter jejuni. One isolate (RM4183) which was designated as Campylobacter jejuni was found to group with Campylobacter coli and also appears to actually be Campylobacter coli by full MLST sequencing.

Example 11: Identification of *Acinetobacter baumannii* Using Broad Range Survey and Division-Wide Primers in Epidemiological Surveillance

[412] To test the capability of the broad range survey and division-wide primer sets of Table 5 in identification of Acinetobacter species, 183 clinical samples were obtained from individuals participating in, or in contact with individuals participating in Operation Iraqi Freedom (including US service personnel, US civilian patients at the Walter Reed Army Institute of Research (WRAIR), medical staff, Iraqi civilians and enemy prisoners. In addition, 34 environmental samples were obtained from hospitals in Iraq, Kuwait, Germany, the United States and the USNS Comfort, a hospital ship.

[413] Upon amplification of nucleic acid obtained from the clinical samples, primer pairs 346-349, 360, 361, 354, 362 and 363 (Table 5) all produced bacterial bioagent amplicons which identified Acinetobacter baumannii in 215 of 217 samples. The organism Klebsiella pneumoniae was identified in the remaining two samples. In addition, 14 different strain types (containing single nucleotide polymorphisms relative to a reference strain of Acinetobacter baumannii) were identified and assigned

arbitrary numbers from 1 to 14. Strain type 1 was found in 134 of the sample isolates and strains 3 and 7 were found in 46 and 9 of the isolates respectively.

[414] The epidemiology of strain type 7 of Acinetobacter baumannii was investigated. Strain 7 was found in 4 patients and 5 environmental samples (from field hospitals in Iraq and Kuwait). The index patient infected with strain 7 was a pre-war patient who had a traumatic amputation in March of 2003 and was treated at a Kuwaiti hospital. The patient was subsequently transferred to a hospital in Germany and then to WRAIR. Two other patients from Kuwait infected with strain 7 were found to be non-infectious and were not further monitored. The fourth patient was diagnosed with a strain 7 infection in September of 2003 at WRAIR. Since the fourth patient was not related involved in Operation Iraqi Freedom, it was inferred that the fourth patient was the subject of a noscoomial infection acquired at WRAIR as a result of the spread of strain 7 from the index patient.

[415] The epidemiology of strain type 3 of Acinetobacter baumannii was also investigated. Strain type 3 was found in 46 samples, all of which were from patients (US service members, Iraqi civilians and enemy prisoners) who were treated on the USNS Comfort hospital ship and subsequently returned to Iraq or Kuwait. The occurrence of strain type 3 in a single locale may provide evidence that at least some of the infections at that locale were a result of nosocomial infections.

[416] This example thus illustrates an embodiment of the present invention wherein the methods of analysis of bacterial bioagent identifying amplicons provide the means for epidemiological surveillance.

Example 12: Selection and Use of Triangulation Genotyping Analysis Primer Pairs for Acinetobacter haumanii

[417] To combine the power of high-throughput mass spectrometric analysis of bioagent identifying amplicons with the sub-species characteristic resolving power provided by triangulation genotyping analysis, an additional 21 primer pairs were selected based on analysis of housekeeping genes of the genus Acinetobacter. Genes to which the drill-down triangulation genotyping analysis primers hybridize for production of bacterial bioagent identifying amplicons include anthranilate synthase component I (trpE), adenylate kinase (adk), adenine glycosylase (mutY), fumarate hydratase (fumC), and pyrophosphate phospho-hydratase (ppa). These 21 primer pairs are indicated with reference to sequence listings in Table 14. Primer pair numbers 1151-1154 hybridize to and amplify segments of ture. Primer pair numbers 1155-1167 hybridize to and amplify segments of mutY. Primer pair numbers 1165-1170 hybridize to and amplify segments of fumC. Primer pair numbers a segment of ppa.

Primer pair numbers: 2846-2848 hybridize to and amplify segments of the parC gene of DNA topoisomerase which include a codon known to confer quinolone drug resistance upon sub-types of Acinetobacter baumannii. Primer pair numbers 2852-2854 hybridize to and amplify segments of the gyrA gene of DNA gyrase which include a codon known to confer quinolone drug resistance upon subtypes of Acinetobacter baumannii. Primer pair numbers 2922 and 2972 are speciating primers which are useful for identifying different species members of the genus Acinetobacter. The primer names given in Table 14A (with the exception of primer pair numbers 2846-2848, 2852-2854) indicate the coordinates to which the primers hybridize to a reference sequence which comprises a concatenation of the genes TrpE, efp (elongation factor p), adk, mutT, fumC, and ppa. For example, the forward primer of primer pair 1151 is named AB MLST-11-OIF007_62_91_F because it hybridizes to the Actnetobacter primer reference sequence of strain type 11 in sample 007 of Operation Iraqi Freedom (OIF) at positions 62 to 91. DNA was sequenced from strain type 11 and from this sequence data and an artificial concatenated sequence of partial gene extractions was assembled for use in design of the triangulation genotyping analysis primers. The stretches of arbitrary residues "N"s in the concatenated sequence were added for the convenience of separation of the partial gene extractions (40N for AB_MLST (SEQ ID NO: 1444)).

[418] The hybridization coordinates of primer pair numbers 2846-2848 are with respect to GenBank Accession number X95819. The hybridization coordinates of primer pair numbers 2852-2854 are with respect to GenBank Accession number AY642140. Sequence residue "I" appearing in the forward and reverse primers of primer pair number 2972 represents inosine.

Table 14A: Triangulation Genotyping Analysis Primer Pairs for Identification of Sub-species characteristics (Strain Type) of Members of the Bacterial Genus *Acinetobacter*

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)
1151	AB MLST-11-01F007 62 91 F	454	AB MLST-11-01F007_169_203_R	1418
1152	AB MLST-11-01F007 185 214 P	243	AB_MLST-11-0IF007_291_324_R	969
1153	AB_MLST-11-01F007_260_289_F	541	AB_MLST-11-01F007_364_393_R	1400
1154	AB MLST-11-01F007_206_239_F	436	AB_MLST-11-01F007_318_344_R	1036
1155	AB MLST-11-01F007_522_552_F	378	AB_MLST-11-01F007_587_610_R	1392
1156	AB MLST-11-01F007_547_571_F	250	AB_MLST-11-01F007_656_686_R	902
1157	AB_MLST-11-01F007 601 627 F	256	AB MLST-11-01F007_710_736_R	881
1158	AB_MLST-11-01F007_1202_1225_F	384	AB MLST-11-01F007 1266 1296 R	878
1159	AB_MLST-11-01F007_1202_1225_F	384	AB MLST-11-01F007 1299 1316 R	1199
1160	AB MLST-11-01F007 1234 1264 F	694	AB MLST-11-01F007 1335_1362_R	1215

1161	AB_MLST-11-01F007_1327_1356_F	225	AB MLST-11-01F007 1422 1448 R	1212
1162	AB_MLST-11-01F007_1345_1369_F	383	AB_MLST-11-01F007_1470_1494_R	1083
1163	AB_MLST-11-01F007_1351_1375_F	662	AB_MLST-11-01F007_1470_1494_R	1083
1164	AB MLST-11-01F007 1387 1412 F	422	AB MLST-11-01F007 1470 1494 R	1083
1165	AB MLST-11-01F007 1542 1569 F	194	AB MLST-11-01F007 1656 1680 R	1173
1166	AB MLST-11-01F007 1566 1593 F	684	AB MLST-11-01F007 1656 1680 R	1173
				890
1167	AB MLST-11-0IF007 1611 1638 P	375	AB MLST-11-OIF007 1731 1757 R	
1168	AB_MLST-11-01F007_1726_1752_F	182	AB_MLST-11-01F007_1790_1821_R	1195
1169	AB_NLST-11-0IF007_1792_1826_F	656	AB_MLST-11-01F007 1876 1909 R	1151
1170	AB_MLST-11-0IF007_1792_1826_F	656	AB_MLST-11-OIF007_1895_1927_R	1224
1171	AB_MLST-11-01F007_1970_2002_F	618	AB MLST-11-01F007_2097_2118_R	1157
2846	PARC X95819 33 58 F	302	PARC_X95819_121_153_R	852
2847	PARC_X95819_33_58_F	199	PARC X95819 157 178 R	889
2848	PARC X95819 33 58 F	596	PARC X95819 97 128 R	1169
2852	GYRA AY642140 -1 24 F	150	GYRA AY642140 71 100 R	1242
2853	GYRA AY642140 26 54 F	166	GYRA AY642140 121 146 R	1069
2854	GYRA AY642140 26 54 F	166	GYRA AY642140 58 89 R	1168
2922	AB MLST-11-01F007 991 1018 F	583	AB MLST-11-01F007 1110 1137 R	923
2972	AB MLST-11-01F007 1007 1034 F	592	AB MLST-11-01F007 1126 1153 R	924

Table 14B: Triangulation Genotyping Analysis Primer Pairs for Identification of Sub-species characteristics (Strain Type) of Members of the Bacterial Genus Acinetobacter

Primer	Forward Primer		Reverse Primer	
Pair No.	(SEQ ID NO:)	SEQUENCE	(SEQ ID NO:)	SEQUENCE
1151	454	TGAGATTGCTGAACATTTAATGCTGATTGA	1418	TTGTACATTIGAAACAATATGCATGACATGTGAAT
1152	243	TATTGTTTCAAATGTACAAGGTGAAGTGCG	969	TCACAGGTTCTACTTCATCAATAATTTCCATTGC
1153	541	TGGAACGTTATCAGGTGCCCCAAAAATTCG	1400	TTGCAATCGACATATCCATTTCACCATGCC
1154	436	TGAAGTGCSTGATGATATCSATGCACTTGATGTA	1036	TCCGCCAAAAACTCCCCTTTTCACAGG
1155	378	TCGGTTTAGTAAAAGAACGTATTGCTCAACC	1392	TTCTGCTTGAGGAATAGTGCGTGG
1156	250	TCAACCTGACTGCGTGAATGGTTGT	902	TACSTTCTACGATTTCTTCATCAGGTACATC
1157	256	TCAAGCAGAAGCTTTGGAAGAAGAAGG	881	TACAACSTGATAAACACGACCAGAAGC
1158	384	TCGTGCCCGCAATTTGCATAAAGC	878	TAATGCCGGGTAGTGCAATCCATTCTTCTAG
1159	384	TCGTGCCCGCAATTTGCATAAAGC	1199	TGCACCTGCGGTCGAGCG
1160	694	TTGTAGCACAGCAAGGCAAATTTCCTGAAAC	1215	TGCCATCCATAATCACGCCATACTGACG
1161	225	TAGGTTTACGTCAGTATGGCGTGATTATGG	1212	TGCCAGTTTCCACATTTCACGTTCGTG
1162	383	TOGTGATTATGGATGGCAACGTGAA	1083	TOGCTTGAGTGTAGTCATGATTGCG

1163	662	TTATGGATGGCAACGTGAAACGCGT	1083	TCGCTTGAGTGTAGTCATGATTGCG
1164	422	TCTTTGCCATTGAAGATGACTTAAGC	1083	TCGCTTGAGTGTAGTCATGATTGCG
1165	194	TACTAGCGGTAAGCTTAAACAAGATTGC	1173	TGAGTCGGGTTCACTTTACCTGGCA
1166	684	TTGCCAATGATATTOGTTGGTTAGCAAG	1173	TGAGTCGGGTTCACTTTACCTGGCA
1167	375	TCGGCGAAATCCGTATTCCTGAAAATGA	890	TACCGGAAGCACCAGCGACATTAATAG
1158	182	TACCACTATEATGTCGCTGGTGCTTC	1195	TGCAACTGAATAGATTGCAGTAAGTTATAAGC
1169	656	TTATAACTTACIGCAATCIATTCAGTTGGTTGGTG	1151	TGAATTATGCAAGAAGTGATCAATTTTCTCACGA
1170	656	TTATAACTTACTGCAATCTATTCAGTTGCTTGGTG	1224	TGCCGTAACTAACATAAGAGAATTATGCAAGAA
1171	618	TOGTTATGTACCAAATACTTTGTCTGAAGATGG	1157	TGACGGCATCGATACCACCGTC
2846	302	TCCAAAAAATCAGCGCGTACAGTGG	852	TAAAGGATAGGGGTAACTAAATGGCTGAGCCAT
2847	199	TACTTGGTAAATACCACCCACATGGTGA	869	TACCCCAGTTCCCCTGACCTTC
2848	596	TGGTAAATACCACCCACATGGTGAC	1169	TGAGCCATGAGTACCATGGCTTCATAACATGC
2852	150	TARATCTGCCGTGTCGTTGGTGAC	1242	TGCTAAAGTCTTGAGCCATACGAACAATGG
2853	166	TRATOGGTARATATCACCOGCATGGTGAC	1069	TOGATOGAACOGAAGTTACOCTGACC
2854	166	TANTOGGTAANTATCACCOGCATGGTGAC	1168	TGAGCCATACGAACAATGGTTTCATAAACAGC
2922	583	TUGGCGATGCTGCGAAATGGTTAAAAGA	923	TAGTATCACCACGTACACCCGGATCAGT
2972	592	TOOGIGATGCTGCIAAATGGTTAAAAGA	924	TAGTATCACCACGTACICCIGGATCAGT

[419] Analysis of bioagent identifying amplicons obtained using the primers of Table 14B for over 200 samples from Operation Iraqi Freedom resulted in the identification of 50 distinct strain type clusters. The largest cluster, designated strain type 11 (ST11) includes 42 sample isolates, all of which were obtained from US service personnel and Iraqi civilians treated at the 28th Combat Support Hospital in Baghdad. Several of these individuals were also treated on the hospital ship USNS Comfort. These observations are indicative of significant epidemiological correlation/linkage.

[420] All of the sample isolates were tested against a broad panel of antibiotics to characterize their antibiotic resistance profiles. As an example of a representative result from antibiotic susceptibility testing, STI1 was found to consist of four different clusters of isolates, each with a varying degree of sensitivity/resistance to the various antibiotics tested which included penicillins, extended spectrum penicillins, eephalosporins, carbepenem, protein synthesis inhibitors, nucleic acid synthesis inhibitors, anti-metabolites, and anti-cell membrane antibiotics. Thus, the genotyping power of bacterial bioagent identifying amplicons, particularly drill-down bacterial bioagent identifying amplicons, has the potential to increase the understanding of the transmission of infections in combat casualties, to identify the source of infection in the environment, to track hospital transmission of nosocomial infections, and to

rapidly characterize drug-resistance profiles which enable development of effective infection control measures on a time-scale previously not achievable.

Example 13: Triangulation Genotyping Analysis and Codon Analysis of *Acinetobacter baumannii* Samples from Two Health Care Facilities

[421] In this investigation, 88 clinical samples were obtained from Walter Reed Hospital and 95 clinical samples were obtained from Northwestern Medical Center. All samples from both healthcare facilities were suspected of containing sub-types of Acinetobacter baumannii, at least some of which were expected to be resistant to quinolone drugs. Each of the 183 samples was analyzed by the method of the present invention. DNA was extracted from each of the samples and amplified with eight triangulation genotyping analysis primer pairs represented by primer pair numbers: 1151, 1156, 1158. 1160, 1165, 1167, 1170, and 1171. The DNA was also amplified with speciating primer pair number 2922 and codon analysis primer pair numbers 2846-2848 which interrogate a codon present in the parC gene, and primer pair numbers 2852-2854 which bracket a codon present in the gyrA gene. The parC and gyrA codon mutations are both responsible for causing drug resistance in Acinetobacter baumannii. During evolution of drug resistant strains, the gyrA mutation usually occurs before the parC mutation. Amplification products were measured by ESI-TOF mass spectrometry as indicated in Example 4. The base compositions of the amplification products were calculated from the average molecular masses of the amplification products and are shown in Tables 15-18. The entries in each of the tables are grouped according to strain type number, which is an arbitrary number assigned to Acinetobacter baumannii strains in the order of observance beginning from the triangulation genotyping analysis OIF genotyping study described in Example 12. For example, strain type 11 which appears in samples from the Walter Reed Hospital is the same strain as the strain type 11 mentioned in Example 12. Ibis# refers to the order in which each sample was analyzed. Isolate refers to the original sample isolate numbering system used at the location from which the samples were obtained (either Walter Reed Hospital or Northwestern Medical Center). ST = strain type. ND = not detected. Base compositions highlighted with bold type indicate that the base composition is a unique base composition for the amplification product obtained with the pair of primers indicated,

Table 15A: Base Compositions of Amplification Products of 88 A. baumannii Samples Obtained from Walter Reed Hospital and Amplified with Codon Analysis Primer Pairs Targeting the gyrA

Gene

Species	Ibis#	Isolate	ST	PP No: 2852 gyrA	PP No: 2853 gyrA	PP No: 2854 gyrA
A. baumannii	20	1082	1	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. baumannii	13	854	10	A25G23C21T32	A29G28C21T43	A17G13C13T21

A. baumannii	22	1162	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	27	1230	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	31	1367	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	37	1459	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	55	1700	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	64	1777	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	73	1861	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	74	1877	10	ND	A29G28C21T43	A17G13C13T21
A. baumannii	86	1972	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	3	684	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	6	720	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	7	726	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	19	1079	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	21	1123	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	23	1188	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	33	1417	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	34	1431	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii A. baumannii	38 40	1496 1523	11	A25G23C21T32 A25G23C21T32	A29G28C21T43 A29G28C21T43	A17G13C13T21 A17G13C13T21
A. baumannii A. baumannii	42	1640	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	50	1666	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	51	1668	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	52	1695	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	65	1781	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	44	1649	12	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	49A	1658.1	12	A25G23C22T31	A29G28C21T43	A17G13C13T21
A. baumannii	49B	1658.2	12	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	56	1707	12	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	80	1893	12	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	5	693	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	8	749	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	10	839	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	14	865	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	16	888	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	29	1326	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	35	1440	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	41	1524	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	46	1652	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	47	1653	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	48	1657	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	57	1709	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	61	1727	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	63	1762	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	67	1806	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	75	1881	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	77	1886	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	1	649	46	A25G23C21T32	A29G28C21T43	A17G13C13T21

A. baumannii	2	653	46	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	39	1497	16	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	24	1198	15	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	28	1243	15	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	43	1648	15	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	62	1746	15	A25G23C21T32	A29G28C21T43	A17G13C13T21
	4	689	15	A25G23C21T32	A29G28C21T43	A17G13C13T21
			3	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	68	1822			A29G28C21T43	A17G13C13T21
A. baumannii	69	1823A	3	A25G23C21T32		
A. baumannii	70	1823B	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	71	1826	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	72	1860	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	81	1924	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	82	1929	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	85	1966	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	11	841	3	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. baumannii	32	1415	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	45	1651	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	54	1697	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	58	1712	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	60	1725	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	66	1802	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	76	1883	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	78	1891	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	79	1892	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	83	1947	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	84	1964	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	53	1696	24	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. baumannii	36	1458	49	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	59	1716	9	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. baumannii	9	805	30	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. baumannii	18	967	39	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. baumannii	30	1322	48	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. baumannii	26	1218	50	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. sp. 13TU	15	875	A1	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. sp. 13TU	17	895	A1	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. sp. 3	12	853	B7	A25G22C22T32	A30G29C22T40	A17G13C14T20
A. johnsonii	25	1202	NEW1	A25G22C22T32	A30G29C22T40	A17G13C14T20
A. sp. 2082	87	2082	NEW2	A25G22C22T32	A31G28C22T40	A17G13C14T20

Table 15B: Base Compositions Determined from A. baumannii DNA Samples Obtained from Walter Reed Hospital and Amplified with Codon Analysis Primer Pairs Targeting the parC Gene

	Species	Ibis#	Isolate	ST	PP No: 2846 parC	PP No: 2847 parC	PP No: 2848 parC
- 1	A. baumannii	20	1082	1	A33G26C29T33	A29G28C26T31	A16G14C15T15
-	A. baumannii	13	854	10	A33G26C28T34	A29G28C25T32	A16G14C14T16

A. J	baumannii	22	1162	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	27	1230	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
_	baumannii	31	1367	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	37	1459	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	55	1700	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	64	1777	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	73	1861	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	74	1877	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. 1	baumannii	86	1972	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	3	684	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. J	baumannii	6	720	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	7	726	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	19	1079	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	, 21	1123	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	23	1188	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	33	1417	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. J	baumannii	34	1431	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	38	1496	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
-	baumannii	40	1523	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	42	1640	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. 1	baumannii	50	1666	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	51	1668	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	52	1695	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
-	baumannii	65	1781	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. J	baumannii	44	1649	12	A33G26C28T34	A29G28C25T32	A16G14C14T16
_	baumannii	49A	1658.1	12	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	49B	1658.2	12	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	56	1707	12	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. J	baumannii	80	1893	12	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	5	693	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. 1	baumannii	8	749	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. J	baumannii	10	839	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
Α	baumannii	14	865	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. J	baumannii	16	888	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. 1	baumannii	29	1326	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. j	baumannii	35	1440	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. j	baumannii	41	1524	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	46	1652	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. J	baumannii	47	1653	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. J	baumannii	48	1657	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. J	baumannii	57	1709	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	61	1727	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
_	baumannii	63	1762	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	67	1806	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
	baumannii	75	1881	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
_	baumannii	77	1886	14	A33G26C28T34	A29G28C25T32	A16G14C14T16

A. baumannii	2	653	46	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	39	1497	16	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	24	1198	15	A33G26C28T34	A29G29C23T33	A16G14C14T16
A. baumannii	28	1243	15	A33G26C28T34	A29G29C23T33	A16G14C14T16
A. baumannii	43	1648	15	A33G26C28T34	A29G29C23T33	A16G14C14T16
A. baumannii	62	1746	15	A33G26C28T34	A29G29C23T33	A16G14C14T16
A. baumannii	4	689	15	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	68	1822	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	69	1823A	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	70	1823B	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	71	1826	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	72	1860	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	81	1924	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	82	1929	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	85	1966	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	11	841	3	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	32	1415	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	45	1651	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	54	1697	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	58	1712	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	60	1725	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	66	1802	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	76	1883	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	78	1891	24	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	79	1892	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	83	1947	24	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	84	1964	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	53	1696	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	36	1458	49	A34G26C29T32	A30G28C24T32	A16G14C15T15
A. baumannii	59	1716	9	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	9	805	30	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	18	967	39	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	30	1322	48	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	26	1218	50	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. sp. 13TU	15	875	A1	A32G26C28T35	A28G28C24T34	A16G14C15T15
A. sp. 13TU	17	895	A1	A32G26C28T35	A28G28C24T34	A16G14C15T15
A. sp. 3	12	853	B7	A29G26C27T39	A26G32C21T35	A16G14C15T15
A. johnsonii	25	1202	NEW1	A32G28C26T35	A29G29C22T34	A16G14C15T15
A. sp. 2082	87	2082	NEW2	A33G27C26T35	A31G28C20T35	A16G14C15T15

Table 16A: Base Compositions Determined from A. baumannii DNA Samples Obtained from Northwestern Medical Center and Amplified with Codon Analysis Primer Pairs Targeting the gyrA Gene

				PP No: 2852	PP No: 2853	DD No. 2954
Species	Ibis#	Isolate	ST	gyrA	gyrA	gyrA

A. baumannii 54 536 3 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 87 665 3 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 8 80 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 9 91 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 10 92 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 11 131 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 12 137 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 12 137 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 21 21 218 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 26 242 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 94 678 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 1 9 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 2 13 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 3 19 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 3 19 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 4 24 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 5 36 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 6 39 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 13 139 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 15 165 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 15 165 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 17 186 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 17 186 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 17 186 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 24 234 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 25 239 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 24 234 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 27 257 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 24 234 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 24 234 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 25 239 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 37 424 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 37 424 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 37 424							
A. baumannii 8 80 10 A25033C1T33 A29028C21T43 A17013C13T21 A. baumannii 9 91 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 10 92 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 11 131 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 12 137 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 12 137 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 12 137 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 26 242 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 94 678 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 1 9 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 1 9 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 2 13 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 2 13 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 4 24 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 5 36 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 6 39 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 15 165 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 16 170 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 17 186 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 17 186 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 24 234 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 34 389 10 A25033C21T32 A29028C21T43 A17013C13T21 A. baumannii 35 45 1 A	A. baumannii	54	536	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
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A. baumannii 16 170 10 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 17 186 10 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 20 202 10 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 22 221 10 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 24 234 10 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 24 234 10 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 25 239 10 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 24 234 10 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 25 239 10 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 33 370 10 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 34 389 10 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 19 201 14 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 27 257 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 36 422 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 36 422 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 36 422 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 38 434 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 39 473 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 40 482 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 40 482 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 40 482 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 40 516 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 40 516 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 40 516 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17013C13T21	A. baumannii	13	139	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 17 185 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 20 202 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 24 234 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 24 234 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 25 239 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 35 239 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 34 389 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 34 389 10 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 19 201 14 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 29 301 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 29 301 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 37 424 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 37 424 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 38 434 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 38 434 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 38 434 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 40 482 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 44 512 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 40 482 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 40 482 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 40 482 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 45 516 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 50 528 51 A25G23C21T32 A29G28C21T43 A17G13C13T21	A. baumannii	15	165	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 20 202 10 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 22 221 10 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 24 234 10 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 25 239 10 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 25 239 10 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 33 370 10 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 19 201 14 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 19 201 14 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 27 257 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 29 301 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 37 454 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 36 422 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 37 424 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 38 434 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 39 473 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 40 482 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 40 516 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 40 526 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17013C13T21	A. baumannii	16	170	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 22 221 10 A25033C21T32 A29028C21T43 A17G13C13T21 A. baumannii 24 234 10 A25033C21T32 A29028C21T43 A17G13C13T21 A. baumannii 24 234 10 A25033C21T32 A29028C21T43 A17G13C13T21 A. baumannii 33 370 10 A25033C21T32 A29028C21T43 A17G13C13T21 A. baumannii 34 389 10 A25033C21T32 A29028C21T43 A17G13C13T21 A. baumannii 37 257 51 A25033C21T32 A29028C21T43 A17G13C13T21 A. baumannii 27 257 51 A25033C21T32 A29028C21T43 A17G13C13T21 A. baumannii 29 301 51 A25033C21T32 A29028C21T43 A17G13C13T21 A. baumannii 35 422 51 A25033C21T32 A29028C21T43 A17G13C13T21 A. baumannii 36 422 51 A25033C21T32 A29028C21T43 A17G13C13T21 A. baumannii 37 424 51 A25033C21T32 A29028C21T43 A17G13C13T21 A. baumannii 37 424 51 A25033C21T32 A29028C21T43 A17G13C13T21 A. baumannii 39 473 51 A25033C21T32 A29028C21T43 A17G13C13T21 A. baumannii 39 473 51 A25033C21T32 A29028C21T43 A17G13C13T21 A. baumannii 40 482 51 A25033C21T32 A29028C21T43 A17G13C13T21 A. baumannii 40 482 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13T21		17	186	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 24 234 10 A35023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 25 239 10 A35023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 35 3370 10 A35023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 34 389 10 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 19 201 14 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 27 257 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 29 301 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 31 354 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 31 354 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 37 424 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 37 424 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 38 434 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 39 473 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 40 482 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 44 512 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 44 512 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13T21	A. baumannii	20	202	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 25 239 10 A35023C21T32 A29028C21T43 A17013C13T21 A. baumannii 33 370 10 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 34 389 10 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 19 201 14 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 27 257 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 27 257 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 31 354 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 31 354 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 36 422 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 36 422 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 37 424 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 38 434 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 40 482 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 40 482 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 44 512 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 47 522 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 48 526 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 50 538 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 50 538 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 50 550 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17013C13T21		22	221	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 33 370 10 A35033C21T32 A29038C21T43 A17G13C13T21 A. baumannii 34 389 10 A25033C21T32 A29038C21T43 A17G13C13T21 A. baumannii 19 201 14 A25033C21T32 A29038C21T43 A17G13C13T21 A. baumannii 27 257 51 A25033C21T32 A29038C21T43 A17G13C13T21 A. baumannii 29 301 51 A25033C21T32 A29038C21T43 A17G13C13T21 A. baumannii 3 54 51 A25033C21T32 A29038C21T43 A17G13C13T21 A. baumannii 36 422 51 A25033C21T32 A29038C21T43 A17G13C13T21 A. baumannii 37 424 51 A25033C21T32 A29038C21T43 A17G13C13T21 A. baumannii 37 424 51 A25033C21T32 A29038C21T43 A17G13C13T21 A. baumannii 39 473 51 A25033C21T32 A29038C21T43 A17G13C13T21 A. baumannii 39 473 51 A25033C21T32 A29038C21T43 A17G13C13T21 A. baumannii 40 482 51 A25033C21T32 A29038C21T43 A17G13C13T21 A. baumannii 40 482 51 A25033C21T32 A29038C21T43 A17G13C13T21 A. baumannii 45 516 51 A25033C21T32 A29038C21T43 A17G13C13T21 A. baumannii 45 516 51 A25033C21T32 A29038C21T43 A17G13C13T21 A. baumannii 47 522 51 A25033C21T32 A29038C21T43 A17G13C13T21 A. baumannii 50 528 51 A25033C21T32 A29038C21T43 A17G13C13T21 A. baumannii 50 542 51 A25033C21T32 A29038C21T43 A17G13C13T21 A. baumannii 50 542 51 A25033C21T32 A29038C21T43 A17G13C13T21 A.	A. baumannii	24	234	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 34 389 10 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 19 201 14 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 29 301 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 29 301 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 33 354 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 37 424 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 37 424 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 38 434 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 38 434 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 40 482 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 40 482 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 44 512 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21		25	239	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 19 201 14 A25023C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 27 257 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 29 301 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 31 354 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 36 422 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 36 422 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 38 434 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 39 473 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 40 482 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 40 482 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 40 512 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 44 516 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 45 516 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 47 522 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 55 528 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 55 528 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 55 538 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 55 538 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 56 542 51 A25G23C21T32 A29G28C21T43 A17G13C13T21 A. baumannii 64 557 51 A25G23C21T32 A29G28C21T43 A17G13C13T21			370	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 27 257 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 29 301 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 31 354 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 36 422 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 37 424 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 37 424 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 39 473 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 39 473 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 40 482 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 47 522 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 48 526 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 50 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 50 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 50 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 56 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 56 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21		34	389	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 29 301 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 31 354 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 36 422 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 37 424 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 38 434 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 38 434 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 40 482 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 40 482 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 44 512 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 47 522 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 48 526 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 52 531 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 55 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 56 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21	A. baumannii	19	201	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 31 354 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 36 422 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 37 424 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 38 434 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 39 473 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 40 482 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 47 522 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 52 531 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 52 531 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 52 531 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 55 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 55 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 55 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 55 550 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 55 550 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 56 550 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 56 550 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 56 550 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21		27	257	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 36 422 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 37 424 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 38 434 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 39 473 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 40 482 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 47 522 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 50 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 56 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 56 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 56 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21	A. baumannii	29	301	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 37 424 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 38 434 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 39 473 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 40 482 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 44 512 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 47 522 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 48 526 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 53 533 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 56 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 56 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 56 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 66 557 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21			354	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 38 434 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 39 473 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 40 482 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 44 512 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 47 522 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 48 526 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 52 531 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 52 531 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 55 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 55 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 55 550 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 54 556 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21		36	422	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 39 473 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 40 482 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 44 512 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 47 522 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 52 531 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 52 531 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 55 542 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 56 542 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 56 542 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 56 542 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13721		37	424	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 40 482 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 44 512 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 47 522 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 48 526 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 52 531 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 52 531 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 53 533 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 55 542 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 55 542 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 55 550 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13721			434	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 44 512 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 47 522 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 48 526 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 52 531 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 52 531 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 55 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 55 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 59 550 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 59 550 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21			473	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 45 516 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 47 522 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 48 526 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 52 531 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 55 533 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 56 542 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 59 550 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17013C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17013C13T21		40	482	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 47 522 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 48 526 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 52 531 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 53 533 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 56 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 59 550 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 59 550 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21		44	512	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 48 526 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 52 531 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 53 533 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 55 542 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 59 550 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 59 550 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13721	A. baumannii	45	516	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 50 528 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 52 531 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 53 533 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 56 542 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 59 550 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 65 542 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13721 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13721	A. baumannii	47	522	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 52 551 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 53 533 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 56 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 59 550 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 62 556 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21		48	526	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 53 53 53 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 56 542 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 59 550 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 62 556 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21	A. baumannii	50	528	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 56 542 51 A25023C21T32 A29028C21T43 A17033C13721 A. baumannii 59 550 51 A25023C21T32 A29028C21T43 A17033C13721 A. baumannii 62 556 51 A25023C21T32 A29028C21T43 A17033C13721 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17033C13721		52	531	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 59 550 51 A25023C21T32 A29028C21T43 A17613C13721 A. baumannii 62 556 51 A25023C21T32 A29028C21T43 A17613C13721 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17613C13T21		53	533	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 62 556 51 A25023C21T32 A29028C21T43 A17G13C13T21 A. baumannii 64 557 51 A25023C21T32 A29028C21T43 A17G13C13T21			542	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 64 557 51 A25G23C21T32 A29G28C21T43 A17G13C13T21		59	550	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
AI/GISCISIZI			556	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii 70 588 51 A25G23C21T32 A29G28C21T43 A17G13C13T21			557	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
	A. baumannii	70	588	51	A25G23C21T32	A29G28C21T43	A17G13C13T21

			1	. 1		A17G13C13T21
A. baumannii	73	603	51	A25G23C21T32	A29G28C21T43	A17G13C13121
A. baumannii	74	605	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	75	606	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	77	611	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	79	622	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	83	643	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	85	653	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	89	669	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	93	674	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	23	228	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	32	369	52	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	35	393	52	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	30	339	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	41	485	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	42	493	53	A25G23C21T32	A29G28C21T43	A17G13C13121
A. baumannii	43	502	53	A25G23C21T32	A29G28C21T43	
A. baumannii	46	520	53_	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	49	527	53	A25G23C21T32	A29G28C21T43	A17G13C13T21 A17G13C13T21
A. baumannii	51	529	53	A25G23C21T32	A29G28C21T43	
A. baumannii	65	562	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	68	579	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	57	546	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	58	548	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	60	552	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	61	555	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	63	557	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	66	570	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	67	578	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	69	584	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	71	593	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	72	602	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	76	609	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	78	621	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	80	625	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	81	628	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	82	632	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	84	649	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	86	655	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	88	668	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	90	671	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	91	672	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	92	673	54	A25G23C21T32	A29G28C21T43	
A. baumannii	18	196	55	A25G23C22T31	A29G28C21T43	A17G13C13T21
A. baumannii	55	537	27	A25G23C21T32	A29G28C21T43	
A. baumannii	28	263	27	A25G23C22T31	A29G28C22T42	
A. sp. 3	14	164	В7	A25G22C22T32	A30G29C22T40	
mixture	7	71		ND	ND	A17G13C15T19

Table 16B: Base Compositions Determined from A. baumannii DNA Samples Obtained from Northwestern Medical Center and Amplified with Codon Analysis Primer Pairs Targeting the parC Gene

Species	Ibis#	T==1-1-	am.	PP No: 2846	PP No: 2847	PP No: 2848
A. baumannii	54	Isolate 536	ST 3	parC	parC	parC
A. baumannii	87	665	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	8			A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii		80	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	9	91	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	10	92	10	A33G26C28T34	A29G28C25T32	ND
A. baumannii	11	131	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	12	137	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
	21	218	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii A. baumannii	26	242	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
	94	678	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	1	9	_ 10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	2	13	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	3	19	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	4	24	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	5	36	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	6	39	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	13	139	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	15	165	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	16	170	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	17	186	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	20	202	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	22	221	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	24	234	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	25	239	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	33	370	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	34	389	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	19	201	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	27	257	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	29	301	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	31	354	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	36	422	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	37	424	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	38	434	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	39	473	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	40	482	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	44	512	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	45	516	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	47	522	51	A33G26C28T34	A29G28C25T32	A16G14C14T16

A. baumannii	48	526	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	50	528	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	52	531	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	53	533	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	56	542	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	59	550	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	62	556	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	64	557	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	70	588	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	73	603	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	74	605	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	75	606	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	77	611	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	79	622	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	83	643	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
					A29G28C25T32	A16G14C14T16
A. baumannii A. baumannii	85 89	653 669	51 51	A33G26C28T34 A33G26C28T34	A29G28C25T32	A16G14C14T16
	93	674	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
			51	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	23	228				A16G14C13T15
A. baumannii	32	369	52	A34G25C28T34	A30G27C25T32	
A. baumannii	35	393	52	A34G25C28T34	A30G27C25T32	A16G14C14T16
A. baumannii	30	339	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	41	485	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	42	493	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	43	502	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	46	520	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	49	527	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	51	529	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	65	562	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	68	579	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	57	546	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	58	548	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	60	552	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	61	555	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	63	557	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	66	570	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	67	578	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	69	584	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	71	593	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	72	602	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	76	609	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	78	621	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	80	625	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	81	628	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	82	632	54	A33G26C2BT34	A29G28C25T32	A16G14C14T16
A. baumannii	84	649	54	A33G26C2BT34	A29G28C25T32	A16G14C14T16
A. baumannii	86	655	54	A33G26C28T34	A29G28C25T32	A16G14C14T16

A. baumannii	88	668	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	90	671	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	91	672	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	92	673	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	18	196	55	A33G27C28T33	A29G28C25T31	A15G14C15T16
A. baumannii	55	537	27	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	28	263	27	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. sp. 3	14	164	B7	A35G25C29T32	A30G28C17T39	A16G14C15T15
mixture	7	71	-	ND	ND	A17G14C15T14

Table 17A: Base Compositions Determined from A. baumannii DNA Samples Obtained from Walter Reed Hospital and Amplified with Speciating Primer Pair No. 2922 and Triangulation Genotyping Analysis Primer Pair Nos. 1151 and 1156

Genotyping Analysis 1 finder Fair 100s, 1151 and 1150										
Species	Ibis#	Isolate	ST	PP No: 2922 efp	PP No: 1151 trpE	PP No: 1156 Adk				
A. baumannii	20	1082	1	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	13	854	10	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	22	1162	10	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	27	1230	10	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	31	1367	10	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	37	1459	10	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	55	1700	10	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	64	1777	10	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	73	1861	10	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	74	1877	10	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	86	1972	10	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	3	684	11	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	6	720	11	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	7	726	11	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	19	1079	11	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	21	1123	11	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	23	1188	11	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	33	1417	11	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	34	1431	11	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	38	1496	11	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	40	1523	11	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	42	1640	11	A45G34C25T43	A44G35C21T42	A44G32C26T3B				
A. baumannii	50	1666	11	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	51	1668	11	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	52	1695	11	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	65	1781	11	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	44	1649	12	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	49A	1658.1	12	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	49B	1658.2	12	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	56	1707	12	A45G34C25T43	A44G35C21T42	A44G32C26T38				
A. baumannii	80	1893	12	A45G34C25T43	A44G35C21T42	A44G32C26T38				

A. baumannii	5	693	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	8	749	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	10	839	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	14	865	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	16	888	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	29	1326	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	35	1440	14	A44G35C25T43	ND	A44G32C27T37
A. baumannii	41	1524	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	46	1652	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	47	1653	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	48	1657	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	57	1709	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	61	1727	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	63	1762	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	67	1806	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	75	1881	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	77	1886	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii'	1	649	46	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	2	653	46	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	39	1497	16	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	24	1198	15	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	28	1243	15	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	43	1648	15	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	62	1746	15	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	4	689	15	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	68	1822	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	69	1823A	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	70	1823B	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	71	1826	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	72	1860	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	81	1924	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	82	1929	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	85	1966	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	11	841	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	32	1415	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	45	1651	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	54	1697	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	58	1712	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	60	1725	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	66	1802	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	76	1883	24	ND	A43G36C20T43	A44G32C27T37
A. baumannii	78	1891	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	79	1892	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	83	1947	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	84	1964	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	53	1696	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	36	1458	49	A44G35C25T43	A44G35C22T41	A44G32C27T37

A. baumannii	59	1716	9	A44G35C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	9	805	30	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	18	967	39	A45G34C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	30	1322	48	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	26	1218	50	A44G35C25T43	A44G35C21T42	A44G32C26T38
A. sp. 13TU	15	875	A1	A47G33C24T43	A46G32C20T44	A44G33C27T36
A. sp. 13TU	17	895	A1	A47G33C24T43	A46G32C20T44	A44G33C27T36
A. sp. 3	12	853	B7	A46G35C24T42	A42G34C20T46	A43G33C24T40
A. johnsonii	25	1202	NEW1	A46G35C23T43	A42G35C21T44	A43G33C23T41
A. sp. 2082	87	2082	NEW2	A46G36C22T43	A42G32C20T48	A42G34C23T41

Table 17B: Base Compositions Determined from A. baumannii DNA Samples Obtained from Walter Reed Hospital and Amplified with Triangulation Genotyping Analysis Primer Pair Nos.

1158 and 1160 and 1165

				PP No: 1158	PP No: 1160	PP No: 1165
Species	Ibis#	Isolate	ST	mutY	mutY	fumC
A. baumannii	20	1082	1	A27G21C25T22	A32G35C29T33	A40G33C30T36
A. baumannii	13	854	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	22	1162	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	27	1230	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	31	1367	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	37	1459	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	55	1700	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	64	1777	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	73	1861	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	74	1877	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	86	1972	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	3	684	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	6	720	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	7	726	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	19	1079	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	21	1123	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	23	1188	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	33	1417	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	34	1431	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	38	1496	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	40	1523	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	42	1640	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	50	1666	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	51	1668	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	52	1695	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	65	1781	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	44	1649	12	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	49A	1658.1	12	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	49B	1658.2	12	A27G21C26T21	A32G34C29T34	A40G33C30T36

а	baumannii	56	1707	12	A27G21C26T21	A32G34C29T34	A40G33C30T36
Α.	baumannii	80	1893	12	A27G21C26T21	A32G34C29T34	A40G33C30T36
A.	baumannii	5	693	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
Α.	baumannii	8	749	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
Α.	baumannii	10	839	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
Α.	baumannii	14	865	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
Α.	baumannii	16	888	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A.	baumannii	29	1326	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A.	baumannii	35	1440	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
Α.	baumannii	41	1524	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
Α.	baumannii	46	1652	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
А.	baumannii	47	1653	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
Α.	baumannii	48	1657	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
А.	baumannii	57	1709	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
Α.	baumannii	61	1727	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
А.	baumannii	63	1762	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
А.	baumannii	67	1806	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
Α.	baumannii	75	1881	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
А.	baumannii	77	1886	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
Α.	baumannii	1	649	46	A29G19C26T21	A31G35C29T34	A40G33C29T37
А.	baumannii	2	653	46	A29G19C26T21	A31G35C29T34	A40G33C29T37
Α.	baumannii	39	1497	16	A29G19C26T21	A31G35C29T34	A40G34C29T36
Α.	baumannii	24	1198	15	A29G19C26T21	A31G35C29T34	A40G33C29T37
Α.	baumannii	28	1243	15	A29G19C26T21	A31G35C29T34	A40G33C29T37
Α.	baumannii	43	1648	15	A29G19C26T21	A31G35C29T34	A40G33C29T37
Α.	baumannii	62	1746	15	A29G19C26T21	A31G35C29T34	A40G33C29T37
А.	baumannii	4	689	15	A29G19C26T21	A31G35C29T34	A40G33C29T37
A.	baumannii	68	1822	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
А.	baumannii	69	1823A	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
Α.	baumannii	70	1823B	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
Α.	baumannii	71	1826	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
Α.	baumannii	72	1860	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
А.	baumannii	81	1924	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
Α.	baumannii	82	1929	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
А.	baumannii	85	1966	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
А.	baumannii	11	841	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
Α.	baumannii	32	1415	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A.	baumannii	45	1651	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
Α.		54	1697	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
Α.		58	1712	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
Α.	baumannii	60	1725	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
Α.		66	1802	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
Α.	baumannii	76	1883	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
Α.	baumannii	78	1891	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
Α.	baumannii	79	1892	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
Α.	baumannii	83	1947	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
Α.		84	1964	24	A27G21C26T21	A32G35C28T34	A40G33C30T36

A. baumannii	53	1696	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	36	1458	49	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	59	1716	9	A27G21C25T22	A32G35C28T34	A39G33C30T37
A. baumannii	9	805	30	A27G21C25T22	A32G35C28T34	A39G33C30T37
A. baumannii	18	967	39	A27G21C26T21	A32G35C28T34	A39G33C30T37
A. baumannii	30	1322	48	A28G21C24T22	A32G35C29T33	A40G33C30T36
A. baumannii	26	1218	50	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. sp. 13TU	15	875	A1	A27G21C25T22	A30G36C26T37	A41G34C28T36
A. sp. 13TU	17	895	A1	A27G21C25T22	A30G36C26T37	A41G34C28T36
A. sp. 3	12	853	B7	A26G23C23T23	A30G36C27T36	A39G37C26T37
A. johnsonii	25	1202	NEW1	A25G23C24T23	A30G35C30T34	A38G37C26T38
A. sp. 2082	87	2082	NEW2	A26G22C24T23	A31G35C28T35	A42G34C27T36

Table 17C: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Walter Reed Hospital and Amplified with Triangulation Genotyping Analysis Primer Pair Nos.

1167 and 1170 and 1171

Species	Ibis#	Isolate	ST	PP No: 1167 fumC	PP No: 1170 fumC	PP No: 1171 ppa
A. baumannii	20	1082	1	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	13	854	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	22	1162	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	27	1230	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	31	1367	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	37	1459	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	55	1700	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	64	1777	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	73	1861	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	74	1877	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	86	1972	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	3	684	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	6	720	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	7	726	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	19	1079	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	21	1123	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	23	1188	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	33	1417	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	34	1431	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	38	1496	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	40	1523	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	42	1640	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	50	1666	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	51	1668	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	52	1695	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	65	1781	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	44	1649	12	A41G34C34T38	A38G27C21T50	A35G37C33T44

A. baumannii	49A	1658.1	12	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	49B	1658.2	12	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	56	1707	12	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	80	1893	12	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	5	693	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	8	749	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	10	839	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	14	865	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	1.6	888	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	29	1326	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	35	1440	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	41	1524	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	46	1652	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	47	1653	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	48	1657	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii A. baumannii	57 61	1709	14	A40G35C34T38	A38G27C21T50 A38G27C21T50	A35G37C30T47
A. baumannii A. baumannii	63	1727 1762	14	A40G35C34T38 A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	67	1806	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	75	1881	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	77	1886	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	1	649	46	A41G35C32T39	A37G28C20T51	A35G37C32T45
A. baumannii	2	653	46	A41G35C32T39	A37G28C20T51	A35G37C32T45
A. baumannii	39	1497	16	A41G35C32T39	A37G28C20T51	A35G37C30T47
A. baumannii	24	1198	15	A41G35C32T39	A37G28C20T51	A35G37C30T47
A. baumannii	28	1243	15	A41G35C32T39	A37G28C20T51	A35G37C30T47
A. baumannii	43	1648	15	A41G35C32T39	A37G28C20T51	A35G37C30T47
A. baumannii	62	1746	15	A41G35C32T39	A37G28C20T51	A35G37C30T47
A. baumannii	4	689	15	A41G35C32T39	A37G28C20T51	A35G37C30T47
A. baumannii	68	1822	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	69	1823A	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	70	1823B	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	71	1826	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	72	1860	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	81	1924	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	82	1929	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	85	1966	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	11	841	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	32	1415	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	4.5	1651	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	54	1697	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	58	1712	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	60 66	1725	24	A40G35C34T38	A39G26C22T49	A35G37C33T44 A35G37C33T44
A. baumannii A. baumannii	76	1802	24	A40G35C34T38 A40G35C34T38	A39G26C22T49 A39G26C22T49	A35G37C33T44
A. baumannii A. baumannii	76	1883 1891	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	78	1891	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. Daumannii	19	1892	24	A40G35C34T38	A39G26C22T49	1 M35G3/C33T44

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A. baumannii	83	1947	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	84	1964	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	53	1696	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	36	1458	49	A40G35C34T38	A39G26C22T49	A35G37C30T47
A. baumannii	59	1716	9	A40G35C32T40	A38G27C20T51	A36G35C31T47
A. baumannii	9	805	30	A40G35C32T40	A38G27C21T50	A35G36C29T49
A. baumannii	18	967	39	A40G35C33T39	A38G27C20T51	A35G37C30T47
A. baumannii	30	1322	48	A40G35C35T37	A38G27C21T50	A35G37C30T47
A. baumannii	26	1218	50	A40G35C34T38	A38G27C21T50	A35G37C33T44
A. sp. 13TU	15	875	A1	A41G39C31T36	A37G26C24T49	A34G38C31T46
A. sp. 13TU	17	895	A1	A41G39C31T36	A37G26C24T49	A34G38C31T46
A. sp. 3	12	853	B7	A43G37C30T37	A36G27C24T49	A34G37C31T47
A. johnsonii	25	1202	NEW1	A42G38C31T36	A40G27C19T50	A35G37C32T45
A. sp. 2082	87	2082	NEW2	A43G37C32T35	A37G26C21T52	A35G38C31T45

Table 18A: Base Compositions Determined from A. baumannti DNA Samples Obtained from Northwestern Medical Center and Amplified with Speciating Primer Pair No. 2922 and Triangulation Genotyping Analysis Primer Pair Nos. 1151 and 1156

Species	Ibis#	Isolate	ST	PP No: 2922 efp	PP No: 1151 trpE	PP No: 1156 adk
A. baumannii	54	536	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	87	665	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	8	80	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	9	91	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	10	92	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	11	131	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	12	137	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	21	218	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	26	242	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	94	678	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	1.	9	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	2	13	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	3	1.9	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	4	24	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	5	36	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	6	39	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	13	139	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	15	165	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	16	170	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	17	186	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	20	202	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	22	221	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	24	234	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	25	239	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	33	370	10	A45G34C25T43	A44G35C21T42	A44G32C26T38

A. baumannii	34	389	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	19	201	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	27	257	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	29	301	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	31	354	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	36	422	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	37	424	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	38	434	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	39	473	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	40	482	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	44	512	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	45	516	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	47	522	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	48	526	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	50	528	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	52	531	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	53	533	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	56	542	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	59	550	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	62	556	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	64	557	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	70	588	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	73	603	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	74	605	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	75	606	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	77	611	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	79	622	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	83	643	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	85	653	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	89	669	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	93	674	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	23	228	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	32	369	52	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	35	393	52	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	30	339	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	41	485	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	42	493	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	43	502	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	46	520	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	49	527	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	51	529	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	65	562	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	68	579	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	57	546	54	A44G35C25T43	A44G35C20T43	A44G32C2fT38
A. baumannii	58	548	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	60	552	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	61	555	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
		333	7-7	*** ***********************************	A-2033C20143	A24632C20138

A. baumannii	63	557	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	66	570	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	67	578	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	69	584	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	71	593	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	72	602	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	76	609	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	78	621	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	80	625	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	81	628	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	82	632	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	84	649	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	86	655	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
. A. baumannii	88	668	54	A44G35C25T43	A44G35C20T43 .	A44G32C26T38
A. baumannii	90	671	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	91	672	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	92	673	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	18	196	55	A44G35C25T43	A44G35C20T43	A44G32C27T37
A. baumannii	55	537	27	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	28	263	27	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. sp. 3	14	164	B7	A46G35C24T42	A42G34C20T46	A43G33C24T40
mixture	7	71	?	mixture	ND	ND

Table 18B: Base Compositions Determined from A. baumannii DNA Samples Obtained from Northwestern Medical Center and Amplified with Triangulation Genotyping Analysis Primer Pair Nos. 1158, 1160 and 1165

Species	Tbis#	Isolate	ST	PP No: 1158 mutY	PP No: 1160 mutY	PP No: 1165 fumC
A. baumannií	54	536	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	87	665	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	8	80	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	9	91	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	10	92	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	11	131	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	12	137	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	21	218	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	26	242	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	94	678	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	1	9	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	2	13	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	3	19	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	4	24	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	5	36	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	6	39	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	1.3	139	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	15	165	1.0	A27G21C26T21	A32G35C28T34	A40G33C30T36

A. baumannii	16	170	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	17	186	1.0	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	20	202	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	22	221	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	24	234	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	25	239	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	33	370	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	34	389	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	19	201	1.4	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	27	257	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	29	301	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	31	354	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	36	422	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	37	424	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	38	434	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	39	473	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	40	482	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	44	512	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	45	516	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	47	522	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	48	526	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	50	528	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	52	531	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	53	533	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	56	542	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A baumannii	59	550	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	62	556	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	64	557	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	70	588	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	73	603	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	74	605	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	75	606	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	77	611	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	79	622	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	83	643	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	85	653	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	89	669	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	93	674	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	23	228	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	32	369	52	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	35	393	52	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	30	339	53	A28G20C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	41	485	53	A28G20C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	42	493	53	A28G20C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	43	502	53	A28G20C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	46	520	53	A28G20C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	49	527	53	A28G20C26T21	A32G34C29T34	A40G33C30T36

A. baumannii	51	529	53	A28G20C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	65	562	53	A28G20C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	68	579	53	A28G20C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	57	546	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	58	548	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	60	552	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	61	555	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	63	557	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	66	570	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	67	578	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	69	584	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	71	593	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	72	602	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	.76	609	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	78	621	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	80	625	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	81	628	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	82	632	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	84	649	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	86	655	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	88	668	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	90	671	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	91	672	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	92	673	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	18	196	55	A27G21C25T22	A31G36C27T35	A40G33C29T37
A. baumannii	55	537	27	A27G21C25T22	A32G35C28T34	A40G33C30T36
A. baumannii	28	263	27	A27G21C25T22	A32G35C28T34	A40G33C30T36
A. sp. 3	14	164	B7	A26G23C23T23	A30G36C27T36	A39G37C26T37
mixture	7	71	?	ND	ND	ND

Table 18C: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Northwestern Medical Center and Amplified with Triangulation Genotyping Analysis Primer Pair Nos. 1167, 1170 and 1171

Species	Ibis#	Isolate	ST	PP No: 1167 fumC	PP No: 1170 fumC	PP No: 1171 ppa
A. baumannii	54	536	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	87	665	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	8	80	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	9	91	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	10	92	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	11	131	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	12	137	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	21	218	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	26	242	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	94	678	10	A41G34C34T38	A38G27C21T50	A35G37C33T44

A. baumannii	1	9	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	2	13	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	3	19	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	4	24	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	5	36	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	6	39	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	13	139	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	1.5	165	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	16	170	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	17	186	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	20	202	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	22	221	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	24	234	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	25	239	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	33	370	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	34	389	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	19	201	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	27	257	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	29	301	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	31	354	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	36	422	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	37	424	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	38	434	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	39	473	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	40	482	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	44	512	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
	45	516	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii A. baumannii	47	522	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
	48	526	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
	50	528	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	52	531	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii A. baumannii	53	533	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
	56	542	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	59	550	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	62	556	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii A. baumannii	64	557	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
	70	588	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii		603		A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	73		51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	74	605	51		A38G27C21T50	A35G37C30T47
A. baumannii	75	606	51	A40G35C34T38		A35G37C30T47
A. baumannii	77	611	51	A40G35C34T38	A38G27C21T50 A38G27C21T50	A35G37C30T47
A. baumannii	79	622	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	83	643	51	A40G35C34T38		A35G37C30T47
A. baumannii	85	653	51	A40G35C34T38	A38G27C21T50	
A. baumannii	89	669	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	93	674	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	23	228	51	A40G35C34T38	A38G27C21T50	A35G37C30T47

A. baumannii	32	369	52	A40G35C34T38	A38G27C21T50	A35G37C31T46
A. baumannii	35	393	52	A40G35C34T38	A38G27C21T50	A35G37C31T46
A. baumannii	30	339	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
A. baumannii	41	485	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
A. baumannii	42	493	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
A. baumannii	43	502	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
A. baumannii	46	520	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
A. baumannii	49	527	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
A. baumannii	51	529	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
A. baumannii	65	562	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
A. baumannii	68	579	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
A. baumannii	57	546	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	58	548	54	A40G35C34T3B	A39G26C22T49	A35G37C31T46
A. baumannii	60	552	54	A40G35C34T3B	A39G26C22T49	A35G37C31T46
A. baumannii	61	555	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	63	557	54	A40G35C34T3B	A39G26C22T49	A35G37C31T46
A. baumannii	66	570	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	67	578	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	69	584	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	71	593	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	72	602	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	76	609	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A baumannii	78	621	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	80	625	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	81	628	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	82	632	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	84	649	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	86	655	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	88	668	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	90	671	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	91	672	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	92	673	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	18	196	55	A42G34C33T38	A38G27C20T51	A35G37C31T46
A. baumannii	55	537	27	A40G35C33T39	A38G27C20T51	A35G37C33T44
A. baumannii	28	263	27	A40G35C33T39	A38G27C20T51	A35G37C33T44
A. sp. 3	14	164	B7	A43G37C30T37	A36G27C24T49	A34G37C31T47
mixture	7	71	-	ND	ND	ND

[422] Base composition analysis of the samples obtained from Walter Reed hospital indicated that a majority of the strain types identified were the same strain types already characterized by the OIF study of Example 12. This is not surprising since at least some patients from which clinical samples were obtained in OIF were transferred to the Walter Reed Hospital (WRAIR). Examples of these common strain types include: ST10, ST11, ST12, ST14, ST15, ST16 and ST46. A strong correlation was noted between these strain types and the presence of mutations in the gyrA and parC which confer quinolone drug resistance.

[423] In contrast, the results of base composition analysis of samples obtained from Northwestern Medical Center indicate the presence of 4 major strain types: ST10, ST51, ST53 and ST54. All of these strain types have the gyrA quinolone resistance mutation and most also have the parC quinolone resistance mutation, with the exception of ST35. This observation is consistent with the current understanding that the gyrA mutation generally appears before the parC mutation and suggests that the acquisition of these drug resistance mutations is rather recent and that resistant isolates are taking over the wild-type isolates. Another interesting observation was that a single isolate of ST3 (isolate 841) displays a triangulation genotyping analysis pattern similar to other isolates of ST3, but the codon analysis amplification product base compositions indicate that this isolate has not yet undergone the quinolone resistance mutations in gyrA and parC.

[424] The six isolates that represent species other than Acinetobacter baumannii in the samples obtained from the Walter Reed Hospital were each found to not carry the drug resistance mutations.

[425] The results described above involved analysis of 183 samples using the methods and compositions of the present invention. Results were provided to collaborators at the Walter Reed hospital and Northwestern Medical center within a week of obtaining samples. This example highlights the rapid throughput characteristics of the analysis platform and the resolving power of triangulation genotyping analysis and codon analysis for identification of and determination of drug resistance in hosteria

Example 14: Identification of Drug Resistance Genes and Virulence Factors in Staphylococcus aureus

[426] An eight primer pair panel was designed for identification of drug resistance genes and virulence factors of Staphylococcus aureus and is shown in Table 19. The primer sequences are found in Table 2 and are cross-referenced by the primer pair numbers, primer pair names or SEQ ID NOs listed in Table 19.

Table 19: Primer Pairs for Identification of Drug Resistance Genes and Virulence Factors in Staphylococcus aureus

Primer	Forward Primer Name	Forward	Reverse Primer Name	Reverse Primer	Target
Pair		Primer			Gene
No.		(SEQ ID		(SEQ ID	
		NO:)		NO:)	
879	MECA_Y14051_4507_4530_F	288	MECA Y14051 4555 4581 R	1269	mecA
2056	MECI-R_NC003923-41798-	698	MECI-R_NC003923-41798-	1420	MecI-R
	41609_33_60_F		41609_86_113_R		1
2001	PRIMA MCCOCOCCA FERRO	217	PRM3 3200020E2_EE000_	1167	6mma

	56621_366_395_F		56621_438_465_R		1
2086	ERMC_NC005908-2004- 2738_85 116 F	399	ERMC_NC005908-2004- 2738 173 206 R	1041	ermC
2095	PVLUK_NC003923-1529595- 1531285 688 713 F	456	PVLUK_NC003923-1529595- 1531285 775 804 R	1261	Pv-luk
2249	TUFB_NC002758-615038- 616222_696_725_F	430	TUFB_NC002758-615038- 616222_793_820_R	1321	tufB
2256	NUC_NC002758-894288- 894974 316 345 F	174	NUC_NC002758-894288- 894974 396 421 R	853	Nuc
2313	MUPR X75439 2486 2516 F	172	MUPR X75439 2548 2574 R	1360	mupR

- [427] Primer pair numbers 2256 and 2249 are confirmation primers designed with the aim of high level identification of Staphylococcus aureus. The nue gene is a Staphylococcus aureus-specific marker gene. The tufB gene is a universal housekeeping gene but the bioagent identifying amplicon defined by primer pair number 2249 provides a unique base composition (A43 G28 C19 T35) which distinguishes Staphylococcus aureus from other members of the senus staphylococcus aureus from ot
- [428] High level methicillin resistance in a given strain of Staphylococcus aureus is indicated by bioagent identifying amplicons defined by primer pair numbers 879 and 2056. Analyses have indicated that primer pair number 879 is not expected to prime S. sciuri homolog or Enterococcus faecalis/faciem ampicillin-resistant PBP5 homologs.
- [429] Macrolide and erythromycin resistance in a given strain of Staphylococcus aureus is indicated by bioagent identifying amplicons defined by primer pair numbers 2081 and 2086.
- [430] Resistance to mupriocin in a given strain of *Staphylococcus aureus* is indicated by bioagent identifying amplicons defined by primer pair number 2313.
- [431] Virulence in a given strain of Staphylococcus aureus is indicated by bioagent identifying amplicons defined by primer pair number 2095. This primer pair can simultaneously and identify the pvl (lukS-PV) gene and the lukD gene which encodes a homologous enterotoxin. A bioagent identifying amplicon of the lukD gene has a six nucleobase length difference relative to the lukS-PV gene.
- [432] A total of 32 blinded samples of different strains of Staphylococcus aureus were provided by the Center for Disease Control (CDC). Each sample was analyzed by PCR amplification with the eight primer pair panel, followed by purification and measurement of molecular masses of the amplification products by mass spectrometry. Base compositions for the amplification products were calculated. The base compositions provide the information summarized above for each primer pair. The results are shown in Tables 20A and B. One result noted upon un-blinding of the samples is that each of the PVL+ identified in the same samples by standard PCR assays. These results

indicate that the panel of eight primer pairs is useful for identification of drug resistance and virulence sub-species characteristics for *Staphylococcus aureus*. It is expected that a kit comprising one or more of the members of this panel will be a useful embodiment of the present invention.

Table 20A: Drug Resistance and Virulence Identified in Blinded Samples of Various Strains of Staphylococcus aureus with Primer Pair Nos. 2081, 2086, 2095 and 2256

Sample Index No.	Primer Pair No.	Primer Pair No.	Primer Pair No.	Primer Pair No.
	2081 (ermA)	2086 (ermC)	2095 (pv-luk)	2256 (nuc)
CDC0010	-	-	PVL-/lukD+	+
CDC0015	-	-	PVL+/lukD+	+
CDC0019		+	PVL-/lukD+	+
CDC0026	+	-	PVL-/lukD+	+
CDC0030	+	_	PVL-/lukD+	+
CDC004	-	_	PVL+/lukD+	+
CDC0014	•	+	PVL+/lukD+	+
CDC008	-	-	PVL-/lukD+	+
CDC001	+	-	PVL-/lukD+	+
CDC0022	+	-	PVL-/lukD+	+
CDC006	+	-	PVL-/lukD+	+
CDC007	-	-	PVL-/lukD+	+
CDCVRSAL	+	-	PVL-/lukD+	+
CDCVRSA2	+	+	PVL-/lukD+	+
CDC0011	+		PVL-/lukD+	+
CDC0012	-	-	PVL+/lukD-	+
CDC0021	+	-	PVL-/lukD+	+
CDC0023	+	-	PVL-/lukD+	+
CDC0025	+	-	PVL-/lukD+	+
CDC005	-	-	PVL-/lukD+	+
CDC0018	+	-	PVL+/lukD-	+
CDC002	-	-	PVL-/lukD+	+
CDC0028	+	-	PVL-/lukD+	+
CDC003	-	-	PVL-/lukD+	+
CDC0013	-	-	PVL+/lukD+	+
CDC0016	-	-	PVL-/lukD+	+
CDC0027	+	-	PVL-/lukD+	*
CDC0029	-		PVL+/lukD+	+

CDC0020	-	+	PVL-/lukD+	+
CDC0024	-	-	PVL-/lukD+	+
CDC0031	-	-	PVL-/lukD+	+

Table 20B: Drug Resistance and Virulence Identified in Blinded Samples of Various Strains of Staphylococcus aureus with Primer Pair Nos. 2249, 879, 2056, and 2313

Sample	Primer Pair No. 2249	Primer Pair No.	Primer Pair No.	Primer Pair No.
Index No.	(tufB)	879 (mecA)	2056 (mecI-R)	2313 (mupR)
CDC0010	Staphylococcus aureus	+	+	-
CDC0015	Staphylococcus aureus	-	-	-
CDC0019	Staphylococcus aureus	+	+	-
CDC0026	Staphylococcus aureus	+	+	-
CDC0030	Staphylococcus aureus	+	+	-
CDC004	Staphylococcus aureus	+	+	- ,
CDC0014	Staphylococcus aureus	+	+	-
CDC008	Staphylococcus aureus	+	+	-
CDC001	Staphylococcus aureus	+	+	-
CDC0022	Staphylococcus aureus	+	+	-
CDC006	Staphylococcus aureus	+	+	+
CDC007	Staphylococcus aureus	+	+	-
CDCVRSA1	Staphylococcus aureus	+	+	-
CDCVRSA2	Staphylococcus aureus	+	+	-
CDC0011	Staphylococcus aureus	-	-	-
CDC0012	Staphylococcus aureus	+	+	-
CDC0021	Staphylococcus aureus	+	+	-
CDC0023	Staphylococcus aureus	+	+	-
CDC0025	Staphylococcus aureus	+	+	-
CDC005	Staphylococcus aureus	+	+	-
CDC0018	Staphylococcus aureus	+	+	-
CDC002	Staphylococcus aureus	+	+	-
CDC0028	Staphylococcus aureus	+	+	-
CDC003	Staphylococcus aureus	+	+	-
CDC0013	Staphylococcus aureus	+	+	-
CDC0016	Staphylococcus aureus	+	+	-
CDC0027	Staphylococcus aureus	•	+	-
CDC0029	Staphylococcus aureus	+	+	-

	CDC0020	Staphylococcus aureus	-	-	-
		Staphylococcus aureus	+	+	-
1	CDC0024				
		Staphylococcus scleiferi	-	-	-
	CDC0031				

Example 15: Selection and Use of Triangulation Genotyping Analysis Primer Pairs for Staphylococcus aureus

[433] To combine the power of high-throughput mass spectrometric analysis of bioagent identifying amplicons with the sub-species characteristic resolving power provided by triangulation genotyping analysis, a panel of eight triangulation genotyping analysis primer pairs was selected. The primer pairs are designed to produce bioagent identifying amplicons within six different housekeeping genes which are listed in Table 21. The primer sequences are found in Table 2 and are cross-referenced by the primer pair numbers, primer pair names or SEO ID NOs listed in Table 21.

Table 21: Primer Pairs for Triangulation Genotyping Analysis of Staphylococcus aureus

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
2146	ARCC_NC003923-2725050- 2724595 131 161 F	437	ARCC_NC003923-2725050- 2724595_214_245_R	1137	arcc
2149	AROE_NC003923-1674726- 1674277 30 62 F	530	AROB_NC003923-1674726- 1674277 155 181 R	891	aroE
2150	AROE NC003923-1674726- 1674277 204 232 F	474	AROE_NC003923-1674726- 1674277 308 335 R	869	aroE
2156	GMK_NC003923-1190906- 1191334_301_329_F	268	GMK_NC003923-1190906- 1191334_403_432_R	1284	gmk
2157	PTA_NC003923-628885- 629355 237 263 F	418	PTA_NC003923-628885- 629355_314_345_R	1301	pta
2161	TPI_NC003923-830671- 831072 1 34 F	318	TPI_NC003923-830671- 831072 97 129 R	1300	tpi
2163	YQI_NC003923-378916- 379431 142 167 F	440	YQI_NC003923-378916- 379431 259 284 R	1076	yqi
2166	YQI_NC003923-378916- 379431_275_300_F	219	YQI_NC003923-378916- 379431_364_396_R	1013	yqi

[434] The same samples analyzed for drug resistance and virulence in Example 14 were subjected to triangulation genotyping analysis. The primer pairs of Table 21 were used to produce amplification products by PCR, which were subsequently purified and measured by mass spectrometry. Base compositions were calculated from the molecular masses and are shown in Tables 22A and 22B.

Table 22A: Triangulation Genotyping Analysis of Blinded Samples of Various Strains of Staphylococcus aureus with Primer Pair Nos. 2146, 2149, 2150 and 2156

Sample	l	Primer Pair No.	Primer Pair No.	Primer Pair No.	Primer Pair No.
Index No.	Strain	2146 (areC)	2149 (aroE)	2150 (aroE)	2156 (gmk)
CDC0010	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32

	,			·	
CDC0015	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC0019	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC0026	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC0030	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC004	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC0014	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC008	2222	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC001	Mu.50	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31
CDC0022	Mu50	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31
CDC006	Mu50	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31
CDC0011	MRSA252	A45 G24 C18 T28	A58 G24 C19 T51	A41 G36 C12 T43	A51 G29 C21 T31
CDC0012	MRSA252	A45 G24 C18 T28	A58 G24 C19 T51	A41 G36 C12 T43	A51 G29 C21 T31
CDC0021	MRSA252	A45 G24 C18 T28	A58 G24 C19 T51	A41 G36 C12 T43	A51 G29 C21 T31
CDC0023	ST:110	A45 G24 C18 T28	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC0025	ST:110	A45 G24 C18 T28	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC005	ST:338	A44 G24 C18 T29	A59 G23 Cl9 T51	A40 G36 C14 T42	A51 G29 C21 T31
CDC0018	ST:338	A44 G24 C18 T29	A59 G23 C19 T51	A40 G36 C14 T42	A51 G29 C21 T31
CDC002	ST:108	A46 G23 C20 T26	A58 G24 C19 T51	A42 G36 C12 T42	A51 G29 C20 T32
CDC0028	ST:108	A46 G23 C20 T26	A58 G24 C19 T51	A42 G36 C12 T42	A51 G29 C20 T32
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CDC0029	MSSA476	A45 G23 C20 T27	A58 G24 Cl9 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC0020	ST:15	A44 G23 C21 T27	A59 G23 C18 T52	A40 G36 C13 T43	A50 G30 C20 T32
CDC0024	ST:137	A45 G23 C20 T27	A57 G25 C19 T51	A40 G36 C13 T43	A51 G29 C22 T30
CDC0031	***	No product	No product	No product	No product

Table 22B: Triangulation Genotyping Analysis of Blinded Samples of Various Strains of Staphylococcus aureus with Primer Pair Nos. 2146, 2149, 2150 and 2156

Sample		Primer Pair No.	Primer Pair No.	Primer Pair No.	Primer Pair No.
Index No.	Strain	2157 (pta)	2161 (tpi)	2163 (yqi)	2166 (yqi)
CDC0010	COT	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC0015	COL	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC0019	COP	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC0026	COP	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37

_					
CDC0030	COT	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC004	COL	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC0014	COP	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC008	unknown	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC001	16150	A33 G25 C22 T29	A50 G28 C22 T29	A42 036 C22 T43	A36 G31 C19 T36
CDC0022	Mu50	A33 G25 C22 T29	A50 G28 C22 T29	A42 G36 C22 T43	A36 G31 C19 T36
CDC006	Mu50	A33 G25 C22 T29	A50 G28 C22 T29	A42 G36 C22 T43	A36 G31 C19 T36
CDC0011	MRSA252	A32 G25 C23 T29	A50 G28 C22 T29	A42 G36 C22 T43	A37 G30 C18 T37
CDC0012	MRSA252	A32 G25 C23 T29	A50 G28 C22 T29	A42 G36 C22 T43	A37 G30 C18 T37
CDC0021	MRSA252	A32 G25 C23 T29	A50 G28 C22 T29	A42 G36 C22 T43	A37 G30 C18 T37
CDC0023	ST:110	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC0025	ST:110	A32 G25 C23 T29	A51 G28 C22 T28		
CDC005	ST:338	A32 G25 C24 T28	A51 G27 C21 T30	A41 G37 C22 T43 A42 G36 C22 T43	A37 G30 C18 T37
CDC0018	ST:338	A32 G25 C24 T28			A37 G30 C18 T37
CDC002	ST:108	A33 G25 C23 T28	A51 G27 C21 T30	A42 G36 C22 T43	A37 G30 C18 T37
CDC0028	ST:108		A50 G28 C22 T29	A42 G36 C22 T43	A37 G30 C18 T37
		A33 G25 C23 T28	A50 G28 C22 T29	A42 G36 C22 T43	A37 G30 C18 T37
CDC003	ST:107	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC0013	ST:12	A32 G25 C23 T29	A51 G28 C22 T28	A42 G36 C22 T43	A37 G30 C18 T37
CDC0016	ST:120	A32 G25 C24 T28	A50 G28 C21 T30	A42 G36 C22 T43	A37 G30 C18 T37
CDC0027	ST:105	A33 G25 C22 T29	A50 G28 C22 T29	A43 036 C21 T43	A36 G31 C19 T36
CDC0029	MSSA476	A33 G25 C22 T29	A50 G28 C22 T29	A42 G36 C22 T43	A36 G31 C19 T36
CDC0020	ST:15	A33 G25 C22 T29	A50 G28 C21 T30	A42 G36 C22 T43	A36 G31 C18 T37
CDC0024	ST:137	A33 G25 C22 T29	A51 G28 C22 T28	A42 G36 C22 T43	A37 G30 C18 T37
CDC0031	***	A34 G25 C25 T25	A51 G27 C24 T27	No product	No product

[435] Note: *** The sample CDC0031 was identified as Staphylococcus scleiferi as indicated in Example 14. Thus, the triangulation genotyping primers designed for Staphylococcus aureus would generally not be expected to prime and produce amplification products of this organism. Tables 22A and 22B indicate that amplification products are obtained for this organism only with primer pair numbers 2157 and 2161.

[436] A total of thirteen different genotypes of Staphylococcus aureus were identified according to the unique combinations of base compositions across the eight different bioagent identifying amplicons obtained with the eight primer pairs. These results indicate that this eight primer pair panel is useful for analysis of unknown or newly emerging strains of Staphylococcus aureus. It is expected that a kit

comprising one or more of the members of this panel will be a useful embodiment of the present invention.

Example 16: Selection and Use of Triangulation Genotyping Analysis Primer Pairs for Members of the Bacterial Genus *Vibrio*

[437] To combine the power of high-throughput mass spectrometric analysis of bioagent identifying amplicons with the sub-species characteristic resolving power provided by triangulation genotyping analysis, a panel of eight triangulation genotyping analysis primer pairs was selected. The primer pairs are designed to produce bioagent identifying amplicons within seven different housekeeping genes which are listed in Table 23. The primer sequences are found in Table 2 and are cross-referenced by the primer pair numbers, primer pair names or SEQ ID NOs listed in Table 23.

Table 23: Primer Pairs for Triangulation Genotyping Analysis of Members of the Bacterial Genus

Vibrio

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
1098	RNASEP VBC 331 349 F	325	RNASEP VBC 388 414 R	1163	RNAse P
2000	CTXB NC002505 46 70 F	278	CTXB NC002505 132 162 R	1039	ctxB
2001	FUR_NC002505_87_113_F	465	FUR NC002505 205 228 R	1037	fur
2011	GYRB_NC002505_1161_1190 _F	148	GYRB NC002505 1255 1284 R	1172	gyrB
2012	OMPU_NC002505_85_110_F	190	OMPU NC002505 154 180 R	1254	Ucmo
2014	OMPU_NC002505_431_455_F	266	OMPU NC002505 544 567 R	1094	Uamo
2323	CTXA_NC002505-1568114- 1567341_122_149_F	508	CTXA NC002505-1568114- 1567341 186 214 R	1297	ctxA
2927	GAPA NC002505 694 721 F	259	GAPA NC 002505 29 58 R	1060	gapA

[438] A group of 50 bacterial isolates containing multiple strains of both environmental and clinical isolates of Vibrio cholerae, 9 other Vibrio species, and 3 species of Photobacteria were tested using this panel of primer pairs. Base compositions of amplification products obtained with these 8 primer pairs were used to distinguish amongst various species tested, including sub-species differentiation within Vibrio cholerae isolates. For instance, the non-Ol/non-Ol39 isolates were clearly resolved from the Ol and the Ol39 isolates, as were several of the environmental isolates of Vibrio cholerae from the clinical isolates.

[439] It is expected that a kit comprising one or more of the members of this panel will be a useful embodiment of the present invention.

Example 17: Selection and Use of Triangulation Genotyping Analysis Primer Pairs for Members of the Bacterial Genus *Pseudomonas*

[440] To combine the power of high-throughput mass spectrometric analysis of bioagent identifying amplicons with the sub-species characteristic resolving power provided by triangulation genotyping analysis, a panel of twelve triangulation genotyping analysis primer pairs was selected. The primer pairs are designed to produce bioagent identifying amplicons within seven different housekeeping genes which are listed in Table 24. The primer sequences are found in Table 2 and are cross-referenced by the primer pair numbers, primer pair names or SEQ ID NOs listed in Table 24.

Table 24: Primer Pairs for Triangulation Genotyping Analysis of Members of the Bacterial Genus

*Pseudomonas**

Primer	Forward Primer Name	Forward	Reverse Primer Name	Reverse	Target
Pair		Primer		Primer	Gene
No.		(SEQ ID	,	(SEQ ID	
		NO:)		NO:)	
	ACS NC002516-970624-	376	ACS_NC002516-970624-	1265	acsA
2949	971013 299 316 F	i i	971013_364_383_R		
	ARO NC002516-26883-	267	ARO_NC002516-26883-	1341	aroE
2950	27380_4_26_F	1	27380_111_128_R		
	ARO NC002516-26883-	705	ARO NC002516-26883-	1056	aroE
2951	27380_356_377_F		27380_459_484_R		
	GUA_NC002516-4226546-	710	GUA_NC002516-4226546-	1259	guaA
2954	4226174 155 178 F	i i	4226174_265_287_R		1
	GUA_NC002516-4226546-	374	GUA_NC002516-4226546-	1111	guaA
2956	4226174_242_263_F		4226174_355_371_R		
	MUT NC002516-5551158-	545	MUT_NC002516-5551158-	978	mutL
2957	5550717_5_26_F		5550717 99 116 R		
	NUO_NC002516-2984589-	249	NUO_NC002516-2984589-	1095	nuoD
2959	2984954 8 26 F	1	2984954_97_117_R		
	NUO_NC002516-2984589-	195	NUO_NC002516-2984589-	1376	nuoD
2960	2984954_218_239_F	1	2984954_301_326_R		
	PPS NC002516-1915014-	311	PPS_NC002516-1915014-	1014	pps
2961	1915383_44_63_F	1	1915383_140_165_R		
	PPS_NC002516-1915014-	365	PPS_NC002516-1915014-	1052	pps
2962	1915383_240_258_F		1915383_341_360_R		l
	TRP_NC002516-671831-	527	TRP_NC002516-671831-	1071	trpE
2963	672273_24_42_F	1	672273_131_150_R		
	TRP_NC002516-671831-	490	TRP_NC002516-671831-	1182	trpE
2964	672273 261 282 F		672273_362_383_R		l

- [441] It is expected that a kit comprising one or more of the members of this panel will be a useful embodiment of the present invention.
- [442] The present invention includes any combination of the various species and subgeneric groupings falling within the generic disclosure. This invention therefore includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.
- [443] While in accordance with the patent statutes, description of the various embodiments and examples have been provided, the scope of the invention is not to be limited thereto or thereby.

Modifications and alterations of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the present invention.

[444] Therefore, it will be appreciated that the scope of this invention is to be defined by the appended claims, rather than by the specific examples which have been presented by way of example.

[445] Each reference (including, but not limited to, journal articles, U.S. and non-U.S. patents, patent application publications, international patent application publications, gene bank gi or accession numbers, internet web sites, and the like) cited in the present application is incorporated herein by reference in its entirety.

CLAIMS

What is claimed is:

- An oligonucleotide primer 14 to 35 nucleobases in length comprising at least 70% sequence identity with SEO ID NO: 456.
- An oligonucleotide primer 14 to 35 nucleobases in length comprising at least 70% sequence identity with SEO ID NO; 1261.
- A composition comprising the primer of claim 1.
- The composition of claim 3 further comprising an oligonucleotide primer 14 to 35 nucleobases in length comprising at least 70% sequence identity with SEQ ID NO: 1261.
- The composition of claim 4 wherein either or both of said primers comprises at least one
 modified nucleobase.
- The composition of claim 5 wherein said modified nucleobase is 5-propynyluracil or 5propynylcytosine.
- The composition of claim 4 wherein either or both of said primers comprises at least one universal nucleobase.
- 8. The composition of claim 7 wherein said universal nucleobase is inosine.
- The composition of claim 4 wherein either or both of said primers further comprises a nontemplated T residue on the 5'-end.
- 10. The composition of claim 4 wherein either or both of said primers comprises at least one non-template tag.
- 11. The composition of claim 4 wherein either or both of said primers comprises at least one molecular mass modifying tag.
- A kit comprising the composition of claim 4.

13. The kit of claim 12 further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 288:1269, 698:1420, 217:1167, 399:1041, 430:1321, 174:853, and 172:1360.

- 14. The kit of claim 12 further comprising one or more calibration polynucleotides.
- The kit of claim 12 further comprising at least one anion exchange functional group linked to a magnetic bead.
- 16. A method for identification of a strain of Staphylococcus aureus in a sample comprising: amplifying nucleic acid from said strain of Staphylococcus aureus using the composition of claim 4 to obtain an amplification product;
 - determining the molecular mass of said amplification product;
- optionally, determining the base composition of said amplification product from said molecular mass; and
- comparing said molecular mass or said base composition with a plurality of molecular masses or base compositions of known amplification products of strains of Staphylococcus aureus defined by the composition of claim 4, wherein a match between said molecular mass or base composition and a member of said plurality of molecular masses or base compositions identifies said strain of Staphylococcus aureus.
- 17. The method of claim 16 further comprising repeating said amplifying, determining, optionally determining, and comparing steps using at least one additional primer pair, wherein each member of said at least one additional primer pair is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 288:1269, 698:1420, 217:1167, 399:1041, 430:1321, 174:853, and 172:1360.
- 18. The method of claim 16 wherein said strain of Staphylococcus aureus is a virulent strain.
- 19. The method of claim 18 wherein said strain of Staphylococcus aureus is a virulent strain.
- 20. A method for determination of the quantity of a strain of Staphylococcus aureus in a sample comprising:

contacting said sample with the composition of claim 4 and a known quantity of a calibration polynucleotide comprising a calibration sequence;

concurrently amplifying nucleic acid from said a strain of Staphylococcus aureus and nucleic acid from said calibration polynucleotide in said sample with the composition of claim 4 to obtain a first amplification product comprising a bacterial bioagent identifying amplicon and a second amplification product comprising a calibration amplicon;

determining the molecular mass and abundance for said bacterial bioagent identifying amplicon and said calibration amplicon; and

distinguishing said bacterial bioagent identifying amplicon from said calibration amplicon based on molecular mass, wherein comparison of bacterial bioagent identifying amplicon abundance and calibration amplicon abundance indicates the quantity of said strain of Staphylococcus aureus in said sample.

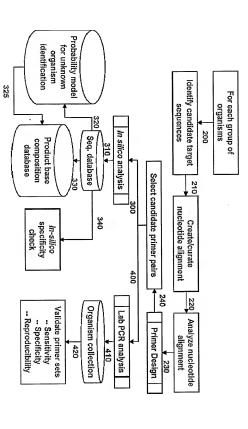
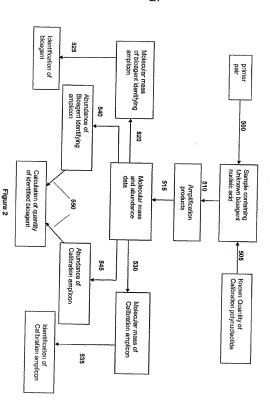


Figure 1



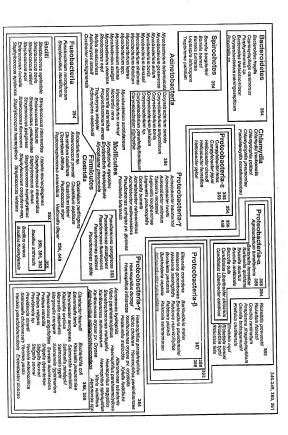


Figure 3

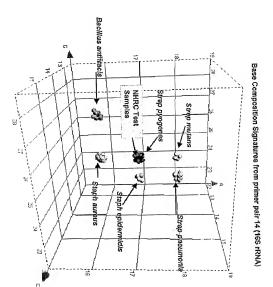
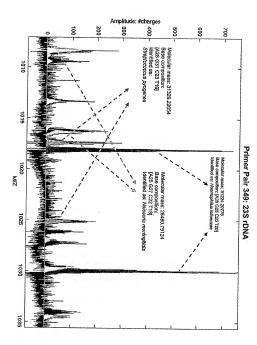


Figure 4

Figure 5



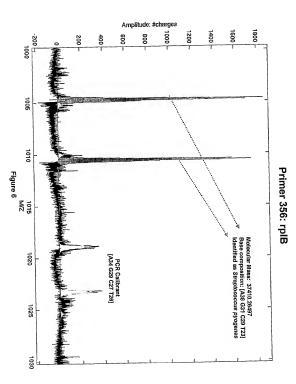
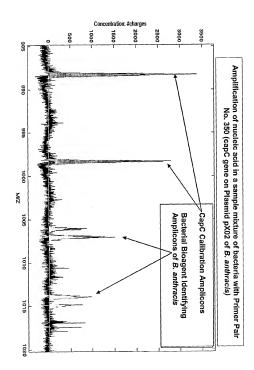


Figure 7



SEQUENCE LISTING

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<223> Calibration Polynucleotide

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<210> 1447
<211> 108
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<212> DNA
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 <212> DNA
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<223> Calibration Polynucleotide
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<210> 1463
<211> 111
<212> DNA
<213> Artificial Sequence
<223> Calibration Sequence
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<223> Combination Calibration Polynucleotide
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